

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
9 March 2006 (09.03.2006)

PCT

(10) International Publication Number
WO 2006/026780 A1(51) International Patent Classification:
C07K 14/33 (2006.01) C07K 14/705 (2006.01)(74) Agents: STATHAKIS, Dean, G. et al.; c/o Allergan, Inc.,
2525 Dupont Drive, Irvine, CA 92612 (US).(21) International Application Number:
PCT/US2005/031613(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.(22) International Filing Date:
1 September 2005 (01.09.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/931,719 1 September 2004 (01.09.2004) US(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).(71) Applicant (*for all designated States except US*): ALLERGAN, INC. [US/US]; 2525 Dupont Drive, Irvine, CA 92715 (US).

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- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DEGRADABLE CLOSTRIDIAL TOXINS

(57) Abstract: The specification discloses modified Clostridial toxins comprising a PAR ligand domain, a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and a Clostridial toxin binding domain; polynucleotide molecules encoding modified Clostridial toxins comprising a PAR ligand domain, a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and a Clostridial toxin binding domain; and method of producing modified Clostridial toxins comprising a PAR ligand domain, a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and a Clostridial toxin binding domain.

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Degradable Clostridial Toxins

[01] This patent application claims priority pursuant to 35 U.S.C. §119(e) to United States provisional patent application Serial No. 60/651494, which was converted on July 5, 2005 from U.S. nonprovisional patent application Serial No. 10/931719 filed Sep. 1, 2004, which is hereby incorporated by reference in its entirety.

[02] All of the patents and publications cited in this application are hereby incorporated by reference in their entirety.

[03] The ability of Clostridial toxins, such as, *e.g.*, Botulinum neurotoxins (BoNTs), BoNT/A, BoNT/B, BoNT/C1, BoNT/D, BoNT/E, BoNT/F and BoNT/G, and Tetanus neurotoxin (TeNT), to inhibit neuronal transmission are being exploited in a wide variety of therapeutic and cosmetic applications, see *e.g.*, William J. Lipham, COSMETIC AND CLINICAL APPLICATIONS OF BOTULINUM TOXIN (Slack, Inc., 2004). As an example, BOTOX® is currently approved in one or more countries for the following indications: achalasia, adult spasticity, anal fissure, back pain, blepharospasm, bruxism, cervical dystonia, essential tremor, glabellar lines or hyperkinetic facial lines, headache, hemifacial spasm, hyperactivity of bladder, hyperhidrosis, juvenile cerebral palsy, multiple sclerosis, myoclonic disorders, nasal labial lines, spasmodic dysphonia, strabismus and VII nerve disorder. In addition, Clostridial toxin therapies are proposed for treating neuromuscular disorders, see *e.g.*, Kei Roger Aoki *et al.*, *Method for Treating Neuromuscular Disorders and Conditions with Botulinum Toxin Types A and B*, U.S. Patent No. 6,872,397 (Mar. 29, 2005); Rhett M. Schiffman, *Methods for Treating Uterine Disorders*, U.S. Patent Publication No. 2004/0175399 (Sep. 9, 2004); Richard L. Barron, *Methods for Treating Ulcers and Gastroesophageal Reflux Disease*, U.S. Patent Publication No. 2004/0086531 (May. 7, 2004); and Kei Roger Aoki, *et al.*, *Method for Treating Dystonia with Botulinum Toxin C to G*, U.S. Patent No. 6,319,505 (Nov. 20, 2001); eye disorders, see *e.g.*, Eric R. First, *Methods and Compositions for Treating Eye Disorders*, U.S. Patent Publication No. 2004/0234532 (Nov. 25, 2004); Kei Roger Aoki *et al.*, *Botulinum Toxin Treatment for Blepharospasm*, U.S. Patent Publication No. 2004/0151740 (Aug. 5, 2004); and Kei Roger Aoki *et al.*, *Botulinum Toxin Treatment for Strabismus*, U.S. Patent Publication No. 2004/0126396 (Jul. 1, 2004); pain, see *e.g.*, Kei Roger Aoki *et al.*, *Pain Treatment by Peripheral Administration of a Neurotoxin*, U.S. Patent No. 6,869,610 (Mar. 22, 2005); Stephen Donovan, *Clostridial Toxin Derivatives and Methods to Treat Pain*, U.S. Patent No. 6,641,820 (Nov. 4, 2003); Kei Roger Aoki, *et al.*, *Method for Treating Pain by Peripheral Administration of a Neurotoxin*, U.S. Patent No. 6,464,986 (Oct. 15, 2002); Kei Roger Aoki and Minglei Cui, *Methods for Treating Pain*, U.S. Patent No. 6,113,915 (Sep. 5, 2000); Martin A. Voet, *Methods for Treating Fibromyalgia*, U.S. Patent 6,623,742 (Sep. 23, 2003); Martin A. Voet, *Botulinum Toxin Therapy for Fibromyalgia*, U.S. Patent Publication No. 2004/0062776 (Apr. 1, 2004); and Kei Roger Aoki *et al.*, *Botulinum Toxin Therapy for Lower Back Pain*, U.S. Patent Publication No. 2004/0037852 (Feb. 26, 2004); muscle injuries, see *e.g.*, Gregory F. Brooks, *Methods for Treating Muscle Injuries*, U.S. Patent No. 6,423,319 (Jul. 23, 2002); headache, see *e.g.*, Martin Voet, *Methods for*

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Treating Sinus Headache, U.S. Patent No. 6,838,434 (Jan. 4, 2005); Kei Roger Aoki *et al.*, *Methods for Treating Tension Headache*, U.S. Patent No. 6,776,992 (Aug. 17, 2004); and Kei Roger Aoki *et al.*, *Method for Treating Headache*, U.S. Patent No. 6,458,365 (Oct. 1, 2002); William J. Binder, *Method for Reduction of Migraine Headache Pain*, U.S. Patent 5,714,469 (Feb. 3, 1998); cardiovascular diseases, see *e.g.*, Gregory F. Brooks and Stephen Donovan, *Methods for Treating Cardiovascular Diseases with Botulinum Toxin*, U.S. Patent No. 6,767,544 (Jul. 27, 2004); neurological disorders, see *e.g.*, Stephen Donovan, *Parkinson's Disease Treatment*, U.S. Patent No. 6,620,415 (Sep. 16, 2003); and Stephen Donovan, *Method for Treating Parkinson's Disease with a Botulinum Toxin*, U.S. Patent No. 6,306,403 (Oct. 23, 2001); neuropsychiatric disorders, see *e.g.*, Stephen Donovan, *Botulinum Toxin Therapy for Neuropsychiatric Disorders*, U.S. Patent Publication No. 2004/0180061 (Sep. 16, 2004); and Steven Donovan, *Therapeutic Treatments for Neuropsychiatric Disorders*, U.S. Patent Publication No. 2003/0211121 (Nov. 13, 2003); endocrine disorders, see *e.g.*, Stephen Donovan, *Method for Treating Endocrine Disorders*, U.S. Patent No. 6,827,931 (Dec. 7, 2004); Stephen Donovan, *Method for Treating Thyroid Disorders with a Botulinum Toxin*, U.S. Patent No. 6,740,321 (May. 25, 2004); Kei Roger Aoki *et al.*, *Method for Treating a Cholinergic Influenced Sweat Gland*, U.S. Patent No. 6,683,049 (Jan. 27, 2004); Stephen Donovan, *Neurotoxin Therapy for Diabetes*, U.S. Patent No. 6,416,765 (Jul. 9, 2002); Stephen Donovan, *Methods for Treating Diabetes*, U.S. Patent No. 6,337,075 (Jan. 8, 2002); Stephen Donovan, *Method for Treating a Pancreatic Disorder with a Neurotoxin*, U.S. Patent No. 6,261,572 (Jul. 17, 2001); Stephen Donovan, *Methods for Treating Pancreatic Disorders*, U.S. Patent No. 6,143,306 (Nov. 7, 2000); cancers, see *e.g.*, Stephen Donovan, *Methods for Treating Bone Tumors*, U.S. Patent No. 6,565,870 (May 20, 2003); Stephen Donovan, *Method for Treating Cancer with a Neurotoxin to Improve Patient Function*, U.S. Patent No. 6,368,605 (Apr. 9, 2002); Stephen Donovan, *Method for Treating Cancer with a Neurotoxin*, U.S. Patent No. 6,139,845 (Oct. 31, 2000); and Mitchell F. Brin and Stephen Donovan, *Methods for Treating Diverse Cancers*, U.S. Patent Publication No. 2005/0031648 (Feb. 10, 2005); otic disorders, see *e.g.*, Stephen Donovan, *Neurotoxin Therapy for Inner Ear Disorders*, U.S. Patent No. 6,358,926 (Mar. 19, 2002); and Stephen Donovan, *Method for Treating Otic Disorders*, U.S. Patent No. 6,265,379 (Jul. 24, 2001); autonomic disorders, see, *e.g.*, Pankaj J. Pasricha and Anthony N. Kalloo, *Method for Treating Gastrointestinal Muscle Disorders and Other Smooth Muscle Dysfunction*, U.S. Patent 5,437,291 (Aug. 1, 1995); as well as other disorders, see *e.g.*, William J. Binder, *Method for Treatment of Skin Lesions Associated with Cutaneous Cell-proliferative Disorders*, U.S. Patent 5,670,484 (Sep. 23, 1997); Eric R. First, *Application of Botulinum Toxin to the Management of Neurogenic Inflammatory Disorders*, U.S. Patent 6,063,768 (May 16, 2000); Marvin Schwartz and Brian J. Freund, *Method to Reduce Hair Loss and Stimulate Hair Growth*, U.S. Patent 6,299,893 (Oct. 9, 2001); Jean D. A. Carruthers and Alastair Carruthers, *Cosmetic Use of Botulinum Toxin for Treatment of Downturned Mouth*, U.S. Patent 6,358,917 (Mar. 19, 2002); Stephen Donovan, *Use of a Clostridial Toxin to Reduce Appetite*, U.S. Patent Publication No. 2004/40253274 (Dec. 16, 2004); and Howard I. Katz and Andrew M. Blumenfeld, *Botulinum Toxin Dental Therapies and Procedures*, U.S. Patent Publication No. 2004/0115139 (Jun. 17, 2004); Kei Roger Aoki, *et al.*, *Treatment of Neuromuscular Disorders and Conditions with Different Botulinum*, U.S. Patent Publication No. 2002/0010138 (Jan. 24, 2002); and Kei

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Roger Aoki, et al., *Use of Botulinum Toxins for Treating Various Disorders and Conditions and Associated Pain*, U.S. Patent Publication No. 2004/0013692 (Jan. 22, 2004). In addition, the expected use of Clostridial toxins, such as, *e.g.*, BoNTs and TeNT, in therapeutic and cosmetic treatments of humans and other mammals is anticipated to expand to an ever widening range of diseases and ailments that can benefit from the properties of these toxins.

[04] Clostridial toxin therapies are successfully used for many indications. Generally, administration of a Clostridial toxin is well tolerated. However, toxin administration in some applications can be challenging because of the larger doses required to achieve a beneficial effect. Larger doses can increase the likelihood that the toxin may move through the interstitial fluids and the circulatory systems, such as, *e.g.*, the cardiovascular system and the lymphatic system, of the body, resulting in the undesirable dispersal of the toxin to areas not targeted for toxin treatment. Such dispersal can lead to undesirable side effects, such as, *e.g.*, inhibition of neurotransmitter release in neurons not targeted for treatment or paralysis of a muscle not targeted for treatment. For example, a patient administered a therapeutically effective amount of a BoNT/A treatment into the neck muscles for torticollis may develop dysphagia because of dispersal of the toxin into the oropharynx. Thus, there remains a need for improved Clostridial toxins that are effective at the site of treatment, but have negligible to minimal effects in areas not targeted for a toxin treatment.

[05] The growing clinical, therapeutic and cosmetic use of Clostridial toxins in therapies requiring larger doses necessitates the pharmaceutical industry to develop modified Clostridial toxins that are effective at the target site of the application, but reduce or prevent the undesirable side-effects associated with the dispersal of the toxins to an unwanted location or locations. The present invention provides novel Clostridial toxins that reduce or prevent unwanted side-effects associated with toxin dispersal into non-targeted areas. These and related advantages are useful for various clinical, therapeutic and cosmetic applications, such as, *e.g.*, the treatment of neuromuscular disorders, neuropathic disorders, eye disorders, pain, muscle injuries, headache, cardiovascular diseases, neuropsychiatric disorders, endocrine disorders, cancers, otic disorders and hyperkinetic facial lines, as well as, other disorders where a Clostridial toxin administration to a mammal can produce a beneficial effect.

BRIEF DESCRIPTION OF THE DRAWINGS

[06] FIG. 1 shows that activated PARs are predominantly targeted toward lysosomes for degradation. PARs are activated by an irreversible mechanism, and once cleaved, most activated PARs are endocytosed and directed, by intracellular trafficking routes, to lysosomes for degradation. Step 1 illustrates cleavage of an inactivated PAR by a protease to unmask the tethered ligand (black box). Step 2 illustrates tethered ligand binding and conformational change of the activated PAR. Step 3 illustrates endocytosis of the activated PAR. Step 4 illustrates the early and late endosomal sorting of the

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internalized activated PAR that result in the trafficking of the receptor to a lysosome. Step 5 illustrates the degradation of the internalized activated PAR by proteases within the lysosome.

[07] FIG. 2 shows modified Clostridial toxins comprising a tethered ligand are targeted toward lysosomes for degradation. Such modified toxins that diffuse into the circulatory system can bind to inactive PARs which triggers endocytosis and the directing of internalized toxins, by intracellular trafficking routes, to lysosomes for degradation. Step 1 illustrates the binding of the modified Clostridial toxin comprising a tethered ligand domain (black box) to a PAR. Step 2 illustrates endocytosis of the toxin-PAR complex. Step 3 illustrates the early and late endosomal sorting of the internalized toxin-PAR complex that result in the trafficking of the complex to a lysosome. Step 5 illustrates the degradation of the internalized toxin-PAR complex by proteases within the lysosome.

[08] FIG. 3 shows a schematic of the current paradigm of neurotransmitter release and Clostridial toxin intoxication in a central and peripheral neuron. FIG. 3a shows a schematic for the neurotransmitter release mechanism of a central and peripheral neuron. The release process can be described as comprising two steps: 1) vesicle docking, where the vesicle-bound SNARE protein of a vesicle containing neurotransmitter molecules associates with the membrane-bound SNARE proteins located at the plasma membrane; and 2) neurotransmitter release, where the vesicle fuses with the plasma membrane and the neurotransmitter molecules are exocytosed. FIG. 3b shows a schematic of the intoxication mechanism for tetanus and botulinum toxin activity in a central and peripheral neuron. This intoxication process can be described as comprising four steps: 1) receptor binding, where a Clostridial toxin binds to a Clostridial receptor system and initiates the intoxication process; 2) complex internalization, where after toxin binding, a vesicle containing the toxin/receptor system complex is endocytosed into the cell; 3) light chain translocation, where multiple events are thought to occur, including, *e.g.*, changes in the internal pH of the vesicle, formation of a channel pore comprising the H_N domain of the Clostridial toxin heavy chain, separation of the Clostridial toxin light chain from the heavy chain, and release of the active light chain and 4) enzymatic target modification, where the activate light chain of Clostridial toxin proteolytically cleaves its target SNARE substrate, such as, *e.g.*, SNAP-25, VAMP or Syntaxin, thereby preventing vesicle docking and neurotransmitter release.

[09] FIG. 4 shows modified Clostridial toxins with a PAR ligand domain located at the amino terminus of the enzymatic domain. FIG. 4A depicts the single polypeptide form of a Clostridial toxin with an amino to carboxyl linear organization comprising a PAR ligand domain, an enzymatic domain, a translocation domain and a binding domain, with the di-chain loop region depicted by the double SS bracket and the resulting di-chain form after di-chain loop cleavage. In this example, a masked PAR ligand domain is located at the amino terminus of the enzymatic domain and a proteolytic cleavage site (P1) is located in front of the PAR ligand binding domain. Upon proteolytic cleavage with a P1 protease, the PAR ligand domain becomes unmasked. P2 is a protease cleavage site used to convert the single chain form of the toxin to the di-chain form. Both P1 and P2 can be a PAR endogenous protease cleavage site or an

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exogenous protease cleavage site and can be cleaved by the same protease or different proteases. FIG. 4B depicts the single polypeptide form of a Clostridial toxin with an amino to carboxyl linear organization comprising a PAR ligand domain, an enzymatic domain, a binding domain and a translocation domain, with the di-chain loop region depicted by the double SS bracket. In this example, a masked PAR ligand domain is located at the amino terminus of the enzymatic domain and a proteolytic cleavage site (P1) is located in front of the PAR ligand binding domain. Upon proteolytic cleavage with a P1 protease, the PAR ligand domain becomes unmasked. P2 is a protease cleavage site used to convert the single chain form of the toxin to the di-chain form. Both P1 and P2 can be a PAR endogenous protease cleavage site or an exogenous protease cleavage site and can be cleaved by the same protease or different proteases. FIG. 4C depicts the single polypeptide form of a Clostridial toxin with an amino to carboxyl linear organization comprising a PAR ligand domain, an enzymatic domain, a translocation domain and a binding domain, with the di-chain loop region depicted by the double SS bracket. In this example, an unmasked PAR ligand domain is located at the amino terminus of the enzymatic domain. P2 is a protease cleavage site used to convert the single chain form of the toxin to the di-chain form and can be a PAR endogenous protease cleavage site or an exogenous protease cleavage site. FIG. 4D depicts the single polypeptide form of a Clostridial toxin with an amino to carboxyl linear organization comprising a PAR ligand domain, an enzymatic domain, a binding domain and a translocation domain, with the di-chain loop region depicted by the double SS bracket. In this example, an unmasked PAR ligand domain is located at the amino terminus of the enzymatic domain. P2 is a protease cleavage site used to convert the single chain form of the toxin to the di-chain form and can be a PAR endogenous protease cleavage site or an exogenous protease cleavage site.

[010] FIG. 5 shows modified Clostridial toxins with a PAR ligand domain located at the amino terminus of the translocation domain. FIG. 5A depicts the single polypeptide form of a Clostridial toxin with an amino to carboxyl linear organization comprising a binding domain, an enzymatic domain, a PAR ligand domain and a translocation domain, with the di-chain loop region depicted by the double SS bracket and the resulting di-chain form after di-chain loop cleavage. In this example, a masked PAR ligand domain is located at the amino terminus of the translocation domain and a proteolytic cleavage site (P1) is located in front of the PAR ligand binding domain. Upon proteolytic cleavage with a P1 protease, the PAR ligand domain becomes unmasked. P1 is also the protease cleavage site used to convert the single chain form of the toxin to the di-chain form. P1 can be a PAR endogenous protease cleavage site or an exogenous protease cleavage site. FIG. 5B depicts the single polypeptide form of a Clostridial toxin with an amino to carboxyl linear organization comprising an enzymatic domain, a PAR ligand domain, a translocation domain and a binding domain, with the di-chain loop region depicted by the double SS bracket. In this example, a masked PAR ligand domain is located at the amino terminus of the translocation domain and a proteolytic cleavage site (P1) is located in front of the PAR ligand binding domain. Upon proteolytic cleavage with a P1 protease, the PAR ligand domain becomes unmasked. P1 is also the protease cleavage site used to convert the single chain form of the toxin to the di-chain form. P1 can be a PAR endogenous protease cleavage site or an exogenous protease cleavage site.

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[011] FIG. 6 shows modified Clostridial toxins with a PAR ligand domain located at the amino terminus of the binding domain. FIG. 6 depicts the single polypeptide form of a Clostridial toxin with an amino to carboxyl linear organization comprising an enzymatic domain, a PAR ligand domain, a binding domain and a translocation domain, with the di-chain loop region depicted by the double SS bracket and the resulting di-chain form after di-chain loop cleavage. In this example, a masked PAR ligand domain is located at the amino terminus of the binding domain and a proteolytic cleavage site (P1) is located in front of the PAR ligand binding domain. Upon proteolytic cleavage with a P1 protease, the PAR ligand domain becomes unmasked. P1 is also the protease cleavage site used to convert the single chain form of the toxin to the di-chain form. P1 can be a PAR endogenous protease cleavage site or an exogenous protease cleavage site.

[012] FIG. 7 shows a plasmid map of prokaryotic expression construct pET29b/BoNT/A-ED-PAR1Tb comprising a polynucleotide molecule of SEQ ID NO: 136 encoding a modified BoNT/A of SEQ ID NO: 85, operably-linked to a carboxyl-terminal polyhistidine binding polypeptide. A Trypsin protease cleavage site is operably-linked between the polyhistidine binding polypeptide and the modified BoNT/A. Abbreviations are as follows: P_{T7}, a bacteriophage T7 promoter region; Thrombin, a polynucleotide molecule encoding a PAR1 Thrombin cleavage site; PAR1-LD, a polynucleotide molecule encoding a PAR1 ligand domain; ED, a polynucleotide molecule encoding a BoNT/A enzymatic domain; TD, a polynucleotide molecule encoding a BoNT/A translocation domain; BD, a polynucleotide molecule encoding a BoNT/A binding domain; Trypsin, a polynucleotide molecule encoding Trypsin cleavage site; 6xHis, a polynucleotide molecule encoding a polyhistidine binding polypeptide; T7 TT, a bacteriophage T7 transcription termination region; f1 origin, a bacteriophage f1 origin of replication; Kanamycin, a polynucleotide molecule encoding an aminophosphotransferase that confers Kanamycin resistance; pBR322 ori, a pBR322 origin of plasmid replication region; lacI, a polynucleotide molecule encoding a lactose I.

[013] FIG. 8 shows a plasmid map of prokaryotic expression construct pET29b/BoNT/A-TD-PAR1Tb comprising a polynucleotide molecule of SEQ ID NO: 144 encoding a modified BoNT/A of SEQ ID NO: 93, operably-linked to a carboxyl-terminal polyhistidine binding polypeptide. A Trypsin protease cleavage site is operably-linked between the polyhistidine binding polypeptide and the modified BoNT/A. Abbreviations are as follows: P_{T7}, a bacteriophage T7 promoter region; ED, a polynucleotide molecule encoding a BoNT/A enzymatic domain; Thrombin, a polynucleotide molecule encoding a PAR1 Thrombin cleavage site; PAR1-LD, a polynucleotide molecule encoding a PAR1 ligand domain; TD, a polynucleotide molecule encoding a BoNT/A translocation domain; BD, a polynucleotide molecule encoding a BoNT/A binding domain; Trypsin, a polynucleotide molecule encoding Trypsin cleavage site; 6xHis, a polynucleotide molecule encoding a polyhistidine binding polypeptide; T7 TT, a bacteriophage T7 transcription termination region; f1 origin, a bacteriophage f1 origin of replication; Kanamycin, a polynucleotide molecule encoding an aminophosphotransferase that confers Kanamycin resistance;

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pBR322 ori, a pBR322 origin of plasmid replication region; lacI, a polynucleotide molecule encoding a lactose I.

[014] FIG. 9 shows a plasmid map of prokaryotic expression construct pET29b/BoNT/A-BD-PAR1Tb comprising a polynucleotide molecule of SEQ ID NO: 152 encoding a modified BoNT/A of SEQ ID NO: 101, operably-linked to a carboxyl-terminal polyhistidine binding polypeptide. A Trypsin protease cleavage site is operably-linked between the polyhistidine binding polypeptide and the modified BoNT/A. Abbreviations are as follows: P_{T7}, a bacteriophage T7 promoter region; Thrombin, a polynucleotide molecule encoding a PAR1 Thrombin cleavage site; PAR1-LD, a polynucleotide molecule encoding a PAR1 ligand domain; ED, a polynucleotide molecule encoding a BoNT/A enzymatic domain; TD, a polynucleotide molecule encoding a BoNT/A translocation domain; BD, a polynucleotide molecule encoding a BoNT/A binding domain; Trypsin, a polynucleotide molecule encoding Trypsin cleavage site; 6xHis, a polynucleotide molecule encoding a polyhistidine binding polypeptide; T7 TT, a bacteriophage T7 transcription termination region; f1 origin, a bacteriophage f1 origin of replication; Kanamycin, a polynucleotide molecule encoding an aminophosphotransferase that confers Kanamycin resistance; pBR322 ori, a pBR322 origin of plasmid replication region; lacI, a polynucleotide molecule encoding a lactose I.

[015] FIG. 10 shows a plasmid map of yeast expression construct pPICZ A/BoNT/A-ED-PAR1Tb comprising a polynucleotide molecule of SEQ ID NO: 136 encoding a modified BoNT/A of SEQ ID NO: 85, operably-linked to carboxyl-terminal c-myc and polyhistidine binding polypeptides. Abbreviations are as follows: P_{AOX1}, an aldehyde oxidase 1 promoter region; Thrombin, a polynucleotide molecule encoding a PAR1 Thrombin cleavage site; PAR1-LD, a polynucleotide molecule encoding a PAR1 ligand domain; ED, a polynucleotide molecule encoding a BoNT/A enzymatic domain; TD, a polynucleotide molecule encoding a BoNT/A translocation domain; BD, a polynucleotide molecule encoding a BoNT/A binding domain; c-myc, a polynucleotide molecule encoding a c-myc binding polypeptide; 6xHis, a polynucleotide molecule encoding a polyhistidine binding polypeptide; AOX1 TT, an aldehyde oxidase 1 transcription termination region; Zeocin™, a polynucleotide molecule encoding a Zeocin™ resistance polypeptide; pUC ori, a pUC origin of plasmid replication region.

[016] FIG. 11 shows a plasmid map of baculovirus transfer construct pBACgus3/BoNT/A-ED-PAR1Tb comprising a polynucleotide molecule of SEQ ID NO: 136 encoding a modified BoNT/A of SEQ ID NO: 85, operably-linked to carboxyl-terminal polyhistidine binding polypeptide. A Thrombin protease cleavage site is operably-linked between the modified BoNT/A and the polyhistidine binding polypeptide. Abbreviations are as follows: P_{PH}, an polyhedrin promoter region; gp64, a polynucleotide molecule encoding a gp64 signal polypeptide; Thrombin, a polynucleotide molecule encoding a PAR1 Thrombin cleavage site; PAR1-LD, a polynucleotide molecule encoding a PAR1 ligand domain; ED, a polynucleotide molecule encoding a BoNT/A enzymatic domain; TD, a polynucleotide molecule encoding a BoNT/A translocation domain; BD, a polynucleotide molecule encoding a BoNT/A binding domain;

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Thrombin, a polynucleotide molecule encoding a Thrombin protease cleavage site; 6xHis, a polynucleotide molecule encoding a polyhistidine binding polypeptide; pUC ori, a pUC origin of plasmid replication region; Ampicillin, a polynucleotide molecule encoding a β -lactamase that confers Ampicillin resistance; f1 ori, a bacteriophage f1 origin of replication; gus, a polynucleotide molecule encoding a β -glucuronidase.

[017] FIG. 12 shows a plasmid map of mammalian expression construct pSecTag2/BoNT/A-ED-PAR1Tb comprising a polynucleotide molecule of SEQ ID NO: 136 encoding a modified BoNT/A of SEQ ID NO: 85, operably-linked to carboxyl-terminal c-myc and polyhistidine binding polypeptides. Abbreviations are as follows: P_{CMV}, an cytomegalovirus promoter region; IgK, a polynucleotide molecule encoding an immunoglobulin K polypeptide; Thrombin, a polynucleotide molecule encoding a PAR1 Thrombin cleavage site; PAR1-LD, a polynucleotide molecule encoding a PAR1 ligand domain; ED, a polynucleotide molecule encoding a BoNT/A enzymatic domain; TD, a polynucleotide molecule encoding a BoNT/A translocation domain; BD, a polynucleotide molecule encoding a BoNT/A binding domain; c-myc, a polynucleotide molecule encoding a c-myc binding polypeptide; 6xHis, a polynucleotide molecule encoding a polyhistidine binding polypeptide; BGH pA, a bovine growth hormone polyadenylation site; f1 ori, a bacteriophage f1 origin of replication; P_{SV40}, a simian virus 40 promoter region; Zeocin™, a region encoding an Zeocin™ resistance polypeptide; pUC ori, a pUC origin of plasmid replication region; Ampicillin, a polynucleotide molecule encoding a β -lactamase that confers Ampicillin resistance.

DETAILED DESCRIPTION

[018] While all details of this process are not yet precisely known, protease-activated G protein-coupled receptor (PAR) signaling elicits responses according to the classic paradigm established for other GPCRs. Although the applicants have no wish to be limited by the following description, the overall signaling mechanism can be described as comprising at least four steps: 1) receptor activation where the protease agonist cleaves a specific site located at the extracellular amino-terminus of the receptor that generates a new amino acid terminus that functions as a tethered ligand; 2) ligand binding where the unmasked tethered ligand binds to the ligand binding domain located in the second extracellular loop of the receptor resulting in a conformational change of the cleaved PAR that promotes intracellular interactions with heteromeric G proteins; 3) signal transduction where, in common with most GPCRs, the PAR-G protein complex signals through various Gq-, Gi- and G $\beta\gamma$ -mediated signaling pathways in a temporal and spatial manner; and 4) signal termination where receptor desensitization and receptor degradation stop the signaling of the activated complex (FIG. 1), see, *e.g.*, Joann Trejo, *Protease-Activated Receptors: New Concepts in Regulation of G Protein-Coupled Receptor Signaling and Trafficking*, 307(2) J. Pharmacol. Exp. Ther. 437-442 (2003); and Valeria S. Ossovskaya and Nigel W. Bennett, *Protease-Activated Receptors: Contribution to Physiology and Disease*, 84(2) Physiol. Rev. 579-621 (2004).

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[019] Despite the irreversible mechanism of receptor activation, signaling initiated by activated PARs appears to be rapidly and efficiently terminated. Signal termination is especially important for regulating the magnitude, duration and fidelity of PAR-elicited cellular responses and appears to be governed by two processes. The first mechanism is receptor desensitization, where enzymatic phosphorylation of the activated PAR by G-protein Receptor Kinases (GRKs) and other kinases uncouple the activated receptor from its associated G proteins and signaling effectors. The second mechanism of PAR-initiated signal termination is receptor degradation, where proteolytic cleavage of the activated PAR by cell-surface proteases on the plasma membrane and by intracellular proteases within lysosomal vesicles destroys the activated receptors. Because of the irreversible nature of PAR activation, internalization of activated PARs and their subsequent sorting to lysosomes appears to be the dominant process for signal termination. Internalization of activated PARs contributes to signal termination both by removing activated receptors from G proteins and signaling effectors and by directing activated receptors to lysosomal vesicles where proteolytic degradation effectively inactivates the activated receptor. In addition to endocytosis of activated receptors, PARs also undergo constitutive endocytosis in the absence of proteolytic activation. Therefore, the unusual and irreversible mode of PAR activation has given rise to a very rapid and efficient means of terminating the signaling events elicited by activated PARs utilizing endocytosis and lysosomal degradation.

[020] The present invention discloses modified Clostridial toxins that can be rapidly removed from the circulatory system by exploiting the processes involved in activated PAR signal termination. Clostridial toxins containing a PAR ligand domain can bind PARs, which initiates the internalization and degradation of such modified toxins. Many tissues of the cardiovascular system and lymphatic system comprise cells which express PARs. In situations where a modified Clostridial toxin comprising a PAR ligand domain has diffused into a circulatory system, this modified toxin can be effectively internalized by a PAR expressing cell and degraded by proteases within lysosomes (FIG. 2). Thus utilizing the processes involved in PAR-elicited signal termination will lessen or remove a Clostridial toxin from the circulatory system thereby reducing or preventing the undesirable side-effects associated with the diffusion of a Clostridial toxin to an unwanted location.

[021] Aspects of the present invention provide modified Clostridial toxins comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain. It is envisioned that the location of the PAR ligand domain in the modified Clostridial toxins of the present specification is located at a free amino terminus, including, without limitation, at the amino terminus of the Clostridial toxin enzymatic domain; at the amino terminus of the Clostridial toxin translocation domain; and at the amino terminus of the Clostridial toxin binding domain. Thus, in embodiments, the modified Clostridial toxins comprise a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin enzymatic domain. In other embodiments, the modified Clostridial toxins comprise a PAR ligand domain; a

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Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin translocation domain. In still other embodiments, the modified Clostridial toxins comprise a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin binding domain.

[022] Other aspects of the present invention provide polynucleotide molecules encoding modified Clostridial toxins comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain. It is envisioned that the location of the PAR ligand domain of the modified Clostridial toxins encoded by polynucleotide molecules of the present specification is located at a free amino terminus, including, without limitation, at the amino terminus of the Clostridial toxin enzymatic domain; at the amino terminus of the Clostridial toxin translocation domain; and at the amino terminus of the Clostridial toxin binding domain. Thus, in embodiments, the polynucleotide molecules encoded modified Clostridial toxins comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin enzymatic domain. In other embodiments, the polynucleotide molecules encoded modified Clostridial toxins comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin translocation domain. In still other embodiments, the polynucleotide molecules encoded modified Clostridial toxins comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin binding domain.

[023] Other aspects of the present invention provide methods of producing a modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain, such method comprising the step of expressing in a cell a polynucleotide molecule encoding a modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain. Other aspects of the present invention provide methods of producing in a cell a modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain, such method comprising the steps of introducing in a cell an expression construct comprising a polynucleotide molecule encoding a modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain and expressing the expression construct in the cell.

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[024] Aspects of the present invention provide, in part, a Clostridial toxin. As used herein, the term "Clostridial toxin" means any polypeptide that can execute the overall cellular mechanism whereby a Clostridial toxin enters a neuron and inhibits neurotransmitter release and encompasses the binding of a Clostridial toxin to a low or high affinity receptor complex, the internalization of the toxin/receptor complex, the translocation of the Clostridial toxin light chain into the cytoplasm and the enzymatic modification of a Clostridial toxin substrate. Clostridia toxins produced by *Clostridium botulinum*, *Clostridium tetani*, *Clostridium baratii* and *Clostridium butyricum* are the most widely used in therapeutic and cosmetic treatments of humans and other mammals. Strains of *C. botulinum* produce seven antigenically-distinct types of Botulinum toxins (BoNTs), which have been identified by investigating botulism outbreaks in man (BoNT/A, /B, /E and /F), animals (BoNT/C1 and /D), or isolated from soil (BoNT/G). BoNTs possess approximately 35% amino acid identity with each other and share the same functional domain organization and overall structural architecture. It is recognized by those of skill in the art that within each type of Clostridial toxin there can be subtypes that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently four BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4, with specific subtypes showing approximately 89% amino acid identity when compared to another BoNT/A subtype. While all seven BoNT serotypes have similar structure and pharmacological properties, each also displays heterogeneous bacteriological characteristics. In contrast, tetanus toxin (TeNT) is produced by a uniform group of *C. tetani*. Two other species of Clostridia, *C. baratii* and *C. butyricum*, also produce toxins similar to BoNT/F and BoNT/E, respectively.

[025] Clostridial toxins are each translated as a single chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulfide loop by a naturally-occurring protease, such as, *e.g.*, an endogenous Clostridial toxin protease or a naturally-occurring proteases produced in the environment. This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by a single disulfide bond and noncovalent interactions. Each mature di-chain molecule comprises three functionally distinct domains: 1) an enzymatic domain located in the LC that includes a metalloprotease region containing a zinc-dependent endopeptidase activity which specifically targets core components of the neurotransmitter release apparatus (Table 1); 2) a translocation domain contained within the amino-terminal half of the HC (H_N) that facilitates release of the LC from intracellular vesicles into the cytoplasm of the target cell (Table 1); and 3) a binding domain found within the carboxyl-terminal half of the HC (H_C) that determines the binding activity and binding specificity of the toxin to the receptor complex located at the surface of the target cell (Table 1).

[026] The binding, translocation and enzymatic activity of these three functional domains are all necessary for toxicity. While all details of this process are not yet precisely known, the overall cellular intoxication mechanism whereby Clostridial toxins enter a neuron and inhibit neurotransmitter release is similar, regardless of type. Although the applicants have no wish to be limited by the following description, the intoxication mechanism can be described as comprising at least four steps: 1) receptor

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binding, 2) complex internalization, 3) light chain translocation, and 4) enzymatic target modification (see FIG. 1). The process is initiated when the H_C domain of a Clostridial toxin binds to a toxin-specific receptor complex located on the plasma membrane surface of a target cell. The binding specificity of a receptor complex is thought to be achieved, in part, by specific combinations of gangliosides and protein receptors that appear to distinctly comprise each Clostridial toxin receptor complex. Once bound, the toxin/receptor complexes are internalized by endocytosis and the internalized vesicles are sorted to specific intracellular routes. The translocation step appears to be triggered by the acidification of the vesicle compartment. This process seems to initiate two important pH-dependent structural rearrangements that increase hydrophobicity and promote formation di-chain form of the toxin. Once activated, light chain endopeptidase of the toxin is released from the intracellular vesicle into the cytosol where it specifically targets one of three known core components of the neurotransmitter release apparatus. These core proteins, vesicle-associated membrane protein (VAMP)/synaptobrevin, synaptosomal-associated protein of 25 kDa (SNAP-25) and Syntaxin, are necessary for synaptic vesicle docking and fusion at the nerve terminal and constitute members of the soluble *N*-ethylmaleimide-sensitive factor-attachment protein-receptor (SNARE) family. BoNT/A and BoNT/E cleave SNAP-25 in the carboxyl-terminal region, releasing a nine or twenty-six amino acid segment, respectively, and BoNT/C1 also cleaves SNAP-25 near the carboxyl-terminus. The botulinum serotypes BoNT/B, BoNT/D, BoNT/F and BoNT/G, and tetanus toxin, act on the conserved central portion of VAMP, and release the amino-terminal portion of VAMP into the cytosol. BoNT/C1 cleaves syntaxin at a single site near the cytosolic membrane surface. The selective proteolysis of synaptic SNAREs accounts for the block of neurotransmitter release caused by Clostridial toxins *in vivo*. The SNARE protein targets of Clostridial toxins are common to exocytosis in a variety of non-neuronal types; in these cells, as in neurons, light chain peptidase activity inhibits exocytosis, see, *e.g.*, Yann Humeau *et al.*, *How Botulinum and Tetanus Neurotoxins Block Neurotransmitter Release*, 82(5) *Biochimie*. 427-446 (2000); Kathryn Turton *et al.*, *Botulinum and Tetanus Neurotoxins: Structure, Function and Therapeutic Utility*, 27(11) *Trends Biochem. Sci.* 552-558. (2002); Giovanna Lalli *et al.*, *The Journey of Tetanus and Botulinum Neurotoxins in Neurons*, 11(9) *Trends Microbiol.* 431-437, (2003).

Table 1. Clostridial Toxin Reference Sequences and Regions				
Toxin	SEQ ID NO.	LC	H _N	H _C
BoNT/A	1	M1-K448	A449-K871	N872-L1296
BoNT/B	2	M1-K441	A442-S858	E859-E1291
BoNT/C1	3	M1-K449	T450-N866	N867-E1291
BoNT/D	4	M1-R445	D446-N862	S863-E1276
BoNT/E	5	M1-R422	K423-K845	R846-K1252
BoNT/F	6	M1-K439	A440-K864	K865-E1274
BoNT/G	7	M1-K446	S447-S863	N864-E1297
TeNT	8	M1-A457	S458-V879	I880-D1315

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[027] A Clostridial toxin includes, without limitation, naturally occurring Clostridial toxin variants, such as, *e.g.*, Clostridial toxin isoforms and Clostridial toxin subtypes; non-naturally occurring Clostridial toxin variants, such as, *e.g.*, conservative Clostridial toxin variants, non-conservative Clostridial toxin variants, Clostridial toxin chimeric variants and active Clostridial toxin fragments thereof, or any combination thereof. As used herein, the term "Clostridial toxin variant," whether naturally-occurring or non-naturally-occurring, means a Clostridial toxin that has at least one amino acid change from the corresponding region of the disclosed reference sequences (see Table 1) and can be described in percent identity to the corresponding region of that reference sequence. As non-limiting examples, a BoNT/A variant comprising amino acids 1-1296 of SEQ ID NO: 1 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1296 of SEQ ID NO: 1; a BoNT/B variant comprising amino acids 1-1291 of SEQ ID NO: 2 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1291 of SEQ ID NO: 2; a BoNT/C1 variant comprising amino acids 1-1291 of SEQ ID NO: 3 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1291 of SEQ ID NO: 3; a BoNT/D variant comprising amino acids 1-1276 of SEQ ID NO: 4 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1276 of SEQ ID NO: 4; a BoNT/E variant comprising amino acids 1-1252 of SEQ ID NO: 5 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1252 of SEQ ID NO: 5; a BoNT/F variant comprising amino acids 1-1274 of SEQ ID NO: 6 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1274 of SEQ ID NO: 6; a BoNT/G variant comprising amino acids 1-1297 of SEQ ID NO: 7 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1297 of SEQ ID NO: 7; and a TeNT variant comprising amino acids 1-1315 of SEQ ID NO: 8 will have at least one amino acid difference, such as, *e.g.*, an amino acid substitution, deletion or addition, as compared to the amino acid region 1-1315 of SEQ ID NO: 8.

[028] Any of a variety of sequence alignment methods can be used to determine percent identity, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[029] Global methods align sequences from the beginning to the end of the molecule and determine the best alignment by adding up scores of individual residue pairs and by imposing gap penalties. Non-limiting methods include, *e.g.*, CLUSTAL W, see, *e.g.*, Julie D. Thompson et al., *CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice*, 22(22) Nucleic Acids Research 4673-4680 (1994); and

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iterative refinement, see, *e.g.*, Osamu Gotoh, *Significant Improvement in Accuracy of Multiple Protein Sequence Alignments by Iterative Refinement as Assessed by Reference to Structural Alignments*, 264(4) J. Mol. Biol. 823-838 (1996).

[030] Local methods align sequences by identifying one or more conserved motifs shared by all of the input sequences. Non-limiting methods include, *e.g.*, Match-box, see, *e.g.*, Eric Depiereux and Ernest Feytmans, *Match-Box: A Fundamentally New Algorithm for the Simultaneous Alignment of Several Protein Sequences*, 8(5) CABIOS 501-509 (1992); Gibbs sampling, see, *e.g.*, C. E. Lawrence *et al.*, *Detecting Subtle Sequence Signals: A Gibbs Sampling Strategy for Multiple Alignment*, 262(5131) Science 208-214 (1993); Align-M, see, *e.g.*, Ivo Van Walle *et al.*, *Align-M – A New Algorithm for Multiple Alignment of Highly Divergent Sequences*, 20(9) Bioinformatics, 1428-1435 (2004).

[031] Hybrid methods combine functional aspects of both global and local alignment methods. Non-limiting methods include, *e.g.*, segment-to-segment comparison, see, *e.g.*, Burkhard Morgenstern *et al.*, *Multiple DNA and Protein Sequence Alignment Based On Segment-To-Segment Comparison*, 93(22) Proc. Natl. Acad. Sci. U.S.A. 12098-12103 (1996); T-Coffee, see, *e.g.*, Cédric Notredame *et al.*, *T-Coffee: A Novel Algorithm for Multiple Sequence Alignment*, 302(1) J. Mol. Biol. 205-217 (2000); MUSCLE, see, *e.g.*, Robert C. Edgar, *MUSCLE: Multiple Sequence Alignment With High Score Accuracy and High Throughput*, 32(5) Nucleic Acids Res. 1792-1797 (2004); and DIALIGN-T, see, *e.g.*, Amarendran R Subramanian *et al.*, *DIALIGN-T: An Improved Algorithm for Segment-Based Multiple Sequence Alignment*, 6(1) BMC Bioinformatics 66 (2005).

[032] As used herein, the term “naturally occurring Clostridial toxin variant” means any Clostridial toxin produced without the aid of any human manipulation, including, without limitation, Clostridial toxin isoforms produced from alternatively-spliced transcripts, Clostridial toxin isoforms produced by spontaneous mutation and Clostridial toxin subtypes. Non-limiting examples of a Clostridial toxin isoform include, *e.g.*, BoNT/A isoforms, BoNT/B isoforms, BoNT/C1 isoforms, BoNT/D isoforms, BoNT/E isoforms, BoNT/F isoforms, BoNT/G isoforms, and TeNT isoforms. Non-limiting examples of a Clostridial toxin subtype include, *e.g.*, BoNT/A subtypes BoNT/A1, BoNT/A2, BoNT/A3 and BoNT/A4; BoNT/B subtypes BoNT/B1, BoNT/B2, BoNT/B bivalent and BoNT/B nonproteolytic; BoNT/C1 subtypes BoNT/C1-1 and BoNT/C1-2; BoNT/E subtypes BoNT/E1, BoNT/E2 and BoNT/E3; and BoNT/F subtypes BoNT/F1, BoNT/F2, BoNT/F3 and BoNT/F4.

[033] As used herein, the term “non-naturally occurring Clostridial toxin variant” means any Clostridial toxin produced with the aid of human manipulation, including, without limitation, Clostridial toxins produced by genetic engineering using random mutagenesis or rational design and Clostridial toxins produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin variants include, *e.g.*, conservative Clostridial toxin variants, non-conservative Clostridial toxin variants, Clostridial toxin chimeric variants and active Clostridial toxin fragments.

[034] As used herein, the term “conservative Clostridial toxin variant” means a Clostridial toxin that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference Clostridial toxin sequence (Table 1). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin variant can function in substantially the same manner as the reference Clostridial toxin on which the conservative Clostridial toxin variant is based, and can be substituted for the reference Clostridial toxin in any aspect of the present invention. A conservative Clostridial toxin variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the reference Clostridial toxin on which the conservative Clostridial toxin variant is based. A conservative Clostridial toxin variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin on which the conservative Clostridial toxin variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin on which the conservative Clostridial toxin variant is based. Non-limiting examples of a conservative Clostridial toxin variant include, *e.g.*, conservative BoNT/A variants, conservative BoNT/B variants, conservative BoNT/C1 variants, conservative BoNT/D variants, conservative BoNT/E variants, conservative BoNT/F variants, conservative BoNT/G variants, and conservative TeNT variants.

[035] As used herein, the term “non-conservative Clostridial toxin variant” means a Clostridial toxin in which 1) at least one amino acid is deleted from the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based; 2) at least one amino acid added to the reference Clostridial toxin on which the non-conservative Clostridial toxin is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin sequence (Table 1). A non-conservative Clostridial toxin variant can function in substantially the same manner as the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based, and can be substituted for the reference Clostridial toxin in any aspect of the present invention. A non-conservative Clostridial toxin variant can delete one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids from the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based. A non-conservative Clostridial toxin variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids to the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based. A non-conservative

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Clostridial toxin variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, ten or more amino acids, 20 or more amino acids, 30 or more amino acids, 40 or more amino acids, 50 or more amino acids, 100 or more amino acids, 200 or more amino acids, 300 or more amino acids, 400 or more amino acids, or 500 or more amino acids from the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based. A non-conservative Clostridial toxin variant can also substitute at least 10 contiguous amino acids, at least 15 contiguous amino acids, at least 20 contiguous amino acids, or at least 25 contiguous amino acids from the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based, that possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference Clostridial toxin on which the non-conservative Clostridial toxin variant is based. Non-limiting examples of a non-conservative Clostridial toxin variant include, *e.g.*, non-conservative BoNT/A variants, non-conservative BoNT/B variants, non-conservative BoNT/C1 variants, non-conservative BoNT/D variants, non-conservative BoNT/E variants, non-conservative BoNT/F variants, non-conservative BoNT/G variants, and non-conservative TeNT variants.

[036] As used herein, the term "Clostridial toxin chimeric variant" means a molecule comprising at least a portion of a Clostridial toxin and at least a portion of at least one other protein to form a toxin with at least one property different from the reference Clostridial toxins of Table 1. Such Clostridial toxin chimeric molecules are described in, *e.g.*, Clifford C. Shone *et al.*, Recombinant Toxin Fragments, US 6,461,617 (Oct. 8, 2002); Keith A. Foster *et al.*, Clostridial Toxin Derivatives Able To Modify Peripheral Sensory Afferent Functions, US 6,395,513 (May 28, 2002); Wei-Jin Lin *et al.*, Neurotoxins with Enhanced Target Specificity, US 2002/0137886 (Sep. 26, 2002); Keith A. Foster *et al.*, Inhibition of Secretion from Non-neural Cells, US 2003/0180289 (Sep. 25, 2003); J. Oliver Dolly *et al.*, Activatable Recombinant Neurotoxins, WO 2001/014570 (Mar. 1, 2001); Clifford C. Shone *et al.*, Recombinant Toxin Fragments, WO 2004/024909 (Mar. 25, 2004); and Keith A. Foster *et al.*, Re-targeted Toxin Conjugates, WO 2005/023309 (Mar. 17, 2005).

[037] It is well documented that toxin molecules can be re-targeted to a cell that is not the toxins' natural target cell. When so re-targeted, these toxins are capable of binding to a desired target cell and, following subsequent translocation into the cytosol, are capable of exerting their effect on the target cell. In this regard, the binding domain is selected so that it will bind to a desired target cell, and allow subsequent passage of the modified Clostridial toxin into an endosome within the target cell. It is envisioned that any non-Clostridial binding domain can be used, including, without limitation, ligands, hormones, growth factors, cytokines, antibodies, antagonists, agonists and reverse-agonists, with the proviso that the non-Clostridial binding domain binds to a cell surface receptor system other than the one used by the Clostridial binding domain of the modified Clostridial toxin. Non-limiting examples of a non-Clostridial binding domain include, growth factors, such as, *e.g.*, Nerve growth factor (NGF), Leukemia inhibitory factor (LIF), Basic fibroblast growth factor (bFGF), Brain-derived neurotrophic factor (BDNF),

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Neurotrophin-3 (NT-3), Hydra head activator peptide (HHAP), Transforming growth factor 1 (TGF-1), Transforming growth factor 2 (TGF-2), Transforming growth factor 3(TGF-3), Epidermal growth factor (EGF) and Ciliary neurotrophic factor (CNTF); cytokines, such as, *e.g.*, Tumor necrosis factor (TNF-), Interleukin-1 (IL-1), Interleukin-1 (IL-1) and Interleukin-8 (IL-8); agonists, such as, *e.g.*, Bradykinin, Dynorphin, β -endorphin, Etorphine, Endomorphin-1, Endomorphin-2, Leu-enkephalin, Met-enkephalin, Galanin, Lofentanil, Nociceptin and an opioid; and antibodies, such as, *e.g.*, antibodies against the lactoseries carbohydrate epitopes found on the surface of dorsal root ganglion neurons (*e.g.* monoclonal antibodies 1B2 and LA4), antibodies against any of the receptors for the ligands given above and antibodies against the surface expressed antigen Thyl (*e.g.* monoclonal antibody MRC OX7). Methods of making and using a Clostridial toxin chimeric variant can comprise a modified Clostridial toxin disclosed in the present specification where the binding domain comprises a non-Clostridial toxin binding domain are described in, *e.g.*, Clifford C. Shone *et al.*, *supra*, (2002); Keith A. Foster *et al.*, *supra*, (2002); Wei-Jin Lin *et al.*, *supra*, (2002); Keith A. Foster *et al.*, *supra*, (2003); J. Oliver Dolly *et al.*, *supra*, (2001); Clifford C. Shone *et al.*, *supra*, (2004); and Keith A. Foster *et al.*, *supra*, (2005).

[038] Thus, in an embodiment, a Clostridial toxin chimeric variant can comprise a modified Clostridial toxin disclosed in the present specification where the binding domain comprises a non-Clostridial toxin binding domain. In aspects of this embodiment, a non-Clostridial toxin binding domain can be, *e.g.*, a ligand, a hormone, a growth factor, a cytokine, an antibody, an opioid, an antagonist, an agonist or a reverse-agonist. In other aspects of this embodiment, a non-Clostridial toxin binding domain is a Nerve growth factor (NGF), a Leukemia inhibitory factor (LIF), a Basic fibroblast growth factor (bFGF), a Brain-derived neurotrophic factor (BDNF), a Neurotrophin-3 (NT-3), a Hydra head activator peptide (HHAP), a Transforming growth factor 1 (TGF-1), a Transforming growth factor 2 (TGF-2), a Transforming growth factor 3(TGF-3), an Epidermal growth factor (EGF) or a Ciliary neurotrophic factor (CNTF). In still other aspects of this embodiment, a non-Clostridial toxin binding domain is a Tumor necrosis factor (TNF-), an Interleukin-1 (IL-1), an Interleukin-1 (IL-1) or an Interleukin-8 (IL-8). In yet other aspects of this embodiment, a non-Clostridial toxin binding domain is a Bradykinin, a Dynorphin, a β -endorphin, an Etorphine, an Endomorphin-1, an Endomorphin-2, a Leu-enkephalin, a Met-enkephalin, a Galanin, a Lofentanil or a Nociceptin. In still other aspects of this embodiment, a non-Clostridial toxin binding domain is an antibody against the lactoseries carbohydrate epitopes found on the surface of dorsal root ganglion neurons (*e.g.* monoclonal antibodies 1B2 and LA4), an antibody against any of the receptors for the binding domains given above or an antibody against the surface expressed antigen Thyl (*e.g.* monoclonal antibody MRC OX7).

[039] It is also envisioned that any of a variety of Clostridial toxin fragments can be useful in aspects of the present invention with the proviso that these active fragments can execute the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. Thus, aspects of this embodiment can include Clostridial toxin fragments having a length of, *e.g.*, at least 300 amino acids, at least 400 amino acids, at least 500 amino acids, at least 600 amino acids, at least 700 amino acids, at

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least 800 amino acids, at least 900 amino acids, at least 1000 amino acids, at least 1100 amino acids and at least 1200 amino acids. Other aspects of this embodiment, can include Clostridial toxin fragments having a length of, *e.g.*, at most 300 amino acids, at most 400 amino acids, at most 500 amino acids, at most 600 amino acids, at most 700 amino acids, at most 800 amino acids, at most 900 amino acids, at most 1000 amino acids, at most 1100 amino acids and at most 1200 amino acids.

[040] It is also envisioned that any of a variety of Clostridial toxin fragments comprising the light chain can be useful in aspects of the present invention with the proviso that these light chain fragments can specifically target the core components of the neurotransmitter release apparatus and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The light chains of Clostridial toxins are approximately 420-460 amino acids in length and comprise an enzymatic domain (Table 1). Research has shown that the entire length of a Clostridial toxin light chain is not necessary for the enzymatic activity of the enzymatic domain. As a non-limiting example, the first eight amino acids of the BoNT/A light chain (residues 1-8 of SEQ ID NO: 1) are not required for enzymatic activity. As another non-limiting example, the first eight amino acids of the TeNT light chain (residues 1-8 of SEQ ID NO: 8) are not required for enzymatic activity. Likewise, the carboxyl-terminus of the light chain is not necessary for activity. As a non-limiting example, the last 32 amino acids of the BoNT/A light chain (residues 417-448 of SEQ ID NO: 1) are not required for enzymatic activity. As another non-limiting example, the last 31 amino acids of the TeNT light chain (residues 427-457 of SEQ ID NO: 8) are not required for enzymatic activity. Thus, aspects of this embodiment can include Clostridial toxin light chains comprising an enzymatic domain having a length of, *e.g.*, at least 350 amino acids, at least 375 amino acids, at least 400 amino acids, at least 425 amino acids and at least 450 amino acids. Other aspects of this embodiment can include Clostridial toxin light chains comprising an enzymatic domain having a length of, *e.g.*, at most 350 amino acids, at most 375 amino acids, at most 400 amino acids, at most 425 amino acids and at most 450 amino acids.

[041] It is also envisioned that any of a variety of Clostridial toxin H_N regions comprising a translocation domain can be useful in aspects of the present invention with the proviso that these active fragments can facilitate the release of the LC from intracellular vesicles into the cytoplasm of the target cell and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The H_N regions from the heavy chains of Clostridial toxins are approximately 410-430 amino acids in length and comprise a translocation domain (Table 1). Research has shown that the entire length of a H_N region from a Clostridial toxin heavy chain is not necessary for the translocating activity of the translocation domain. Thus, aspects of this embodiment can include Clostridial toxin H_N regions comprising a translocation domain having a length of, *e.g.*, at least 350 amino acids, at least 375 amino acids, at least 400 amino acids and at least 425 amino acids. Other aspects of this embodiment can include Clostridial toxin H_N regions comprising translocation domain having a length of, *e.g.*, at most 350 amino acids, at most 375 amino acids, at most 400 amino acids and at most 425 amino acids.

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[042] It is also envisioned that any of a variety of Clostridial toxin H_C regions comprising a binding domain can be useful in aspects of the present invention with the proviso that these active fragments can determine the binding activity and binding specificity of the toxin to the receptor complex located at the surface of the target cell execute the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The H_C regions from the heavy chains of Clostridial toxins are approximately 400-440 amino acids in length and comprise a binding domain (Table 1). Research has shown that the entire length of a H_C region from a Clostridial toxin heavy chain is not necessary for the binding activity of the binding domain. Thus, aspects of this embodiment can include Clostridial toxin H_C regions comprising a binding domain having a length of, *e.g.*, at least 350 amino acids, at least 375 amino acids, at least 400 amino acids and at least 425 amino acids. Other aspects of this embodiment can include Clostridial toxin H_C regions comprising a binding domain having a length of, *e.g.*, at most 350 amino acids, at most 375 amino acids, at most 400 amino acids and at most 425 amino acids.

[043] Thus, in an embodiment, a Clostridial toxin comprises a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and a Clostridial toxin binding domain. In an aspect of this embodiment, a Clostridial toxin comprises a naturally occurring Clostridial toxin variant, such as, *e.g.*, a Clostridial toxin isoform or a Clostridial toxin subtype. In another aspect of this embodiment, a Clostridial toxin comprises a non-naturally occurring Clostridial toxin variant, such as, *e.g.*, a conservative Clostridial toxin variant, a non-conservative Clostridial toxin variant or an active Clostridial toxin fragment, or any combination thereof. In another aspect of this embodiment, a Clostridial toxin comprises a Clostridial toxin enzymatic domain or an active fragment thereof, a Clostridial toxin translocation domain or an active fragment thereof, a Clostridial toxin binding domain or an active fragment thereof, or any combination thereof. In other aspects of this embodiment, a Clostridial toxin can comprise a BoNT/A, a BoNT/B, a BoNT/C1, a BoNT/D, a BoNT/E, a BoNT/F, a BoNT/G or a TeNT.

[044] In another embodiment, a Clostridial toxin comprises a BoNT/A. In an aspect of this embodiment, a BoNT/A comprises a BoNT/A enzymatic domain, a BoNT/A translocation domain and a BoNT/A binding domain. In another aspect of this embodiment, a BoNT/A comprises SEQ ID NO: 1. In another aspect of this embodiment, a BoNT/A comprises a naturally occurring BoNT/A variant, such as, *e.g.*, a BoNT/A isoform or a BoNT/A subtype. In another aspect of this embodiment, a BoNT/A comprises a naturally occurring BoNT/A variant of SEQ ID NO: 1, such as, *e.g.*, a BoNT/A isoform of SEQ ID NO: 1 or a BoNT/A subtype of SEQ ID NO: 1. In still another aspect of this embodiment, a BoNT/A comprises a non-naturally occurring BoNT/A variant, such as, *e.g.*, a conservative BoNT/A variant, a non-conservative BoNT/A variant or an active BoNT/A fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A comprises a non-naturally occurring BoNT/A variant of SEQ ID NO: 1, such as, *e.g.*, a conservative BoNT/A variant of SEQ ID NO: 1, a non-conservative BoNT/A variant of SEQ ID NO: 1 or an active BoNT/A fragment of SEQ ID NO: 1, or any combination thereof. In yet another aspect of this embodiment, a BoNT/A comprises a BoNT/A enzymatic domain or an active fragment thereof, a BoNT/A translocation domain or an active fragment thereof, a BoNT/A binding domain or an active

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fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/A comprising a BoNT/A enzymatic domain of amino acids 1-448 from SEQ ID NO: 1 or an active fragment thereof, a BoNT/A translocation domain of amino acids 449-871 from SEQ ID NO: 1 or an active fragment thereof, a BoNT/A binding domain of amino acids 872-1296 from SEQ ID NO: 1 or an active fragment thereof, and any combination thereof.

[045] In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 1, at least 75% amino acid identity with the SEQ ID NO: 1, at least 80% amino acid identity with SEQ ID NO: 1, at least 85% amino acid identity with SEQ ID NO: 1, at least 90% amino acid identity with SEQ ID NO: 1 or at least 95% amino acid identity with SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 1, at most 75% amino acid identity with the SEQ ID NO: 1, at most 80% amino acid identity with SEQ ID NO: 1, at most 85% amino acid identity with SEQ ID NO: 1, at most 90% amino acid identity with SEQ ID NO: 1 or at most 95% amino acid identity with SEQ ID NO: 1.

[046] In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 1.

[047] In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 1. In yet other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least

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one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 1. In other aspects of this embodiment, a BoNT/A comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 1.

[048] In another embodiment, a Clostridial toxin comprises a BoNT/B. In an aspect of this embodiment, a BoNT/B comprises a BoNT/B enzymatic domain, a BoNT/B translocation domain and a BoNT/B binding domain. In another aspect of this embodiment, a BoNT/B comprises SEQ ID NO: 2. In another aspect of this embodiment, a BoNT/B comprises a naturally occurring BoNT/B variant, such as, *e.g.*, a BoNT/B isoform or a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B comprises a naturally occurring BoNT/B variant of SEQ ID NO: 2, such as, *e.g.*, a BoNT/B isoform of SEQ ID NO: 2 or a BoNT/B subtype of SEQ ID NO: 2. In still another aspect of this embodiment, a BoNT/B comprises a non-naturally occurring BoNT/B variant, such as, *e.g.*, a conservative BoNT/B variant, a non-conservative BoNT/B variant or an active BoNT/B fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B comprises a non-naturally occurring BoNT/B variant of SEQ ID NO: 2, such as, *e.g.*, a conservative BoNT/B variant of SEQ ID NO: 2, a non-conservative BoNT/B variant of SEQ ID NO: 2 or an active BoNT/B fragment of SEQ ID NO: 2, or any combination thereof. In yet another aspect of this embodiment, a BoNT/B comprising a BoNT/B enzymatic domain or an active fragment thereof, a BoNT/B translocation domain or active fragment thereof, a BoNT/B binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a BoNT/B comprising a BoNT/B enzymatic domain of amino acids 1-441 from SEQ ID NO: 2 or active fragment thereof, a BoNT/B translocation domain of amino acids 442-858 from SEQ ID NO: 2 or active fragment thereof, a BoNT/B binding domain of amino acids 859-1291 from SEQ ID NO: 2 or active fragment thereof, and any combination thereof.

[049] In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 2, at least 75% amino acid identity with the SEQ ID NO: 2, at least 80% amino acid identity with SEQ ID NO: 2, at least 85% amino acid identity with SEQ ID NO: 2, at least 90% amino acid identity with SEQ ID NO: 2 or at least 95% amino acid identity with SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 2, at most 75% amino acid identity with the SEQ ID NO: 2, at most 80% amino acid identity with SEQ ID NO: 2, at most 85% amino acid identity with SEQ ID NO: 2, at most 90% amino acid identity with SEQ ID NO: 2 or at most 95% amino acid identity with SEQ ID NO: 2.

[050] In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous

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amino acid substitutions relative to SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 2. In still other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 2.

[051] In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 2. In yet other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 2. In still other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 2. In other aspects of this embodiment, a BoNT/B comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 2.

[052] In another embodiment, a Clostridial toxin comprises a BoNT/C1. In an aspect of this embodiment, a BoNT/C1 comprises a BoNT/C1 enzymatic domain, a BoNT/C1 translocation domain and a BoNT/C1 binding domain. In another aspect of this embodiment, a BoNT/C1 comprises SEQ ID NO: 3. In another aspect of this embodiment, a BoNT/C1 comprises a naturally occurring BoNT/C1 variant, such as, *e.g.*, a BoNT/C1 isoform or a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 comprises a naturally occurring BoNT/C1 variant of SEQ ID NO: 3, such as, *e.g.*, a BoNT/C1 isoform of SEQ ID NO: 3 or a BoNT/C1 subtype of SEQ ID NO: 3. In still another aspect of this embodiment, a BoNT/C1 comprises a non-naturally occurring BoNT/C1 variant, such as, *e.g.*, a conservative BoNT/C1 variant, a non-conservative BoNT/C1 variant or an active BoNT/C1 fragment, or any combination thereof.

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In still another aspect of this embodiment, a BoNT/C1 comprises a non-naturally occurring BoNT/C1 variant of SEQ ID NO: 3, such as, *e.g.*, a conservative BoNT/C1 variant of SEQ ID NO: 3, a non-conservative BoNT/C1 variant of SEQ ID NO: 3 or an active BoNT/C1 fragment of SEQ ID NO: 3, or any combination thereof. In yet another aspect of this embodiment, a BoNT/C1 comprises a BoNT/C1 enzymatic domain or active fragment thereof, a BoNT/C1 translocation domain or active fragment thereof, a BoNT/C1 binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a BoNT/C1 comprises a BoNT/C1 enzymatic domain of amino acid 1-449 from SEQ ID NO: 3 or active fragment thereof, a BoNT/C1 translocation domain of amino acids 450-866 from SEQ ID NO: 3 or active fragment thereof, a BoNT/C1 binding domain of amino acids 867-1291 from SEQ ID NO: 3 or active fragment thereof, and any combination thereof.

[053] In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 3, at least 75% amino acid identity with the SEQ ID NO: 3, at least 80% amino acid identity with SEQ ID NO: 3, at least 85% amino acid identity with SEQ ID NO: 3, at least 90% amino acid identity with SEQ ID NO: 3 or at least 95% amino acid identity with SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 3, at most 75% amino acid identity with the SEQ ID NO: 3, at most 80% amino acid identity with SEQ ID NO: 3, at most 85% amino acid identity with SEQ ID NO: 3, at most 90% amino acid identity with SEQ ID NO: 3 or at most 95% amino acid identity with SEQ ID NO: 3.

[054] In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 3. In still other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 3.

[055] In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 comprises a

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polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 3. In yet other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 3. In still other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 3. In other aspects of this embodiment, a BoNT/C1 comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 3.

[056] In another embodiment, a Clostridial toxin comprises a BoNT/D. In an aspect of this embodiment, a BoNT/D comprises a BoNT/D enzymatic domain, a BoNT/D translocation domain and a BoNT/D binding domain. In another aspect of this embodiment, a BoNT/D comprises SEQ ID NO: 4. In another aspect of this embodiment, a BoNT/D comprises a naturally occurring BoNT/D variant, such as, *e.g.*, a BoNT/D isoform or a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D comprises a naturally occurring BoNT/D variant of SEQ ID NO: 4, such as, *e.g.*, a BoNT/D isoform of SEQ ID NO: 4 or a BoNT/D subtype of SEQ ID NO: 4. In still another aspect of this embodiment, a BoNT/D comprises a non-naturally occurring BoNT/D variant, such as, *e.g.*, a conservative BoNT/D variant, a non-conservative BoNT/D variant or an active BoNT/D fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D comprises a non-naturally occurring BoNT/D variant of SEQ ID NO: 4, such as, *e.g.*, a conservative BoNT/D variant of SEQ ID NO: 4, a non-conservative BoNT/D variant of SEQ ID NO: 4 or an active BoNT/D fragment of SEQ ID NO: 4, or any combination thereof. In yet another aspect of this embodiment, a BoNT/D comprises a BoNT/D enzymatic domain or an active fragment thereof, a BoNT/D translocation domain or an active fragment thereof, a BoNT/D binding domain or an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/D comprising a BoNT/D enzymatic domain of amino acids 1-445 from SEQ ID NO: 4 or an active fragment thereof, a BoNT/D translocation domain of amino acids 446-862 from SEQ ID NO: 4 or an active fragment thereof, a BoNT/D binding domain of amino acids 863-1276 from SEQ ID NO: 4 or an active fragment thereof, and any combination thereof.

[057] In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 4, at least 75% amino acid identity with the SEQ ID NO: 4, at least 80% amino acid identity with SEQ ID NO: 4, at least 85% amino acid identity with SEQ ID NO: 4, at least 90% amino acid identity with SEQ ID NO: 4 or at least 95% amino acid identity with SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 4, at most 75% amino acid identity with the SEQ ID NO: 4, at most

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80% amino acid identity with SEQ ID NO: 4, at most 85% amino acid identity with SEQ ID NO: 4, at most 90% amino acid identity with SEQ ID NO: 4 or at most 95% amino acid identity with SEQ ID NO: 4.

[058] In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 4. In still other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 4.

[059] In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 4. In yet other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 4. In still other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 4. In other aspects of this embodiment, a BoNT/D comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 4.

[060] In another embodiment, a Clostridial toxin comprises a BoNT/E. In an aspect of this embodiment, a BoNT/E comprises a BoNT/E enzymatic domain, a BoNT/E translocation domain and a BoNT/E binding domain. In another aspect of this embodiment, a BoNT/E comprises SEQ ID NO: 5. In another aspect of this embodiment, a BoNT/E comprises a naturally occurring BoNT/E variant, such as, *e.g.*, a BoNT/E

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isoform or a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E comprises a naturally occurring BoNT/E variant of SEQ ID NO: 5, such as, *e.g.*, a BoNT/E isoform of SEQ ID NO: 5 or a BoNT/E subtype of SEQ ID NO: 5. In still another aspect of this embodiment, a BoNT/E comprises a non-naturally occurring BoNT/E variant, such as, *e.g.*, a conservative BoNT/E variant, a non-conservative BoNT/E variant or an active BoNT/E fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E comprises a non-naturally occurring BoNT/E variant of SEQ ID NO: 5, such as, *e.g.*, a conservative BoNT/E variant of SEQ ID NO: 5, a non-conservative BoNT/E variant of SEQ ID NO: 5 or an active BoNT/E fragment of SEQ ID NO: 5, or any combination thereof. In yet another aspect of this embodiment, a BoNT/E comprising a BoNT/E enzymatic domain or an active fragment thereof, a BoNT/E translocation domain or active fragment thereof, a BoNT/E binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a BoNT/E comprising a BoNT/E enzymatic domain of amino acids 1-422 from SEQ ID NO: 5 or active fragment thereof, a BoNT/E translocation domain of amino acids 423-845 from SEQ ID NO: 5 or active fragment thereof, a BoNT/E binding domain of amino acids 846-1252 from SEQ ID NO: 5 or active fragment thereof, and any combination thereof.

[061] In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 5, at least 75% amino acid identity with the SEQ ID NO: 5, at least 80% amino acid identity with SEQ ID NO: 5, at least 85% amino acid identity with SEQ ID NO: 5, at least 90% amino acid identity with SEQ ID NO: 5 or at least 95% amino acid identity with SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 5, at most 75% amino acid identity with the SEQ ID NO: 5, at most 80% amino acid identity with SEQ ID NO: 5, at most 85% amino acid identity with SEQ ID NO: 5, at most 90% amino acid identity with SEQ ID NO: 5 or at most 95% amino acid identity with SEQ ID NO: 5.

[062] In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least one, two,

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three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 5.

[063] In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 5. In still other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/E comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 5.

[064] In another embodiment, a Clostridial toxin comprises a BoNT/F. In an aspect of this embodiment, a BoNT/F comprises a BoNT/F enzymatic domain, a BoNT/F translocation domain and a BoNT/F binding domain. In another aspect of this embodiment, a BoNT/F comprises SEQ ID NO: 6. In another aspect of this embodiment, a BoNT/F comprises a naturally occurring BoNT/F variant, such as, *e.g.*, a BoNT/F isoform or a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F comprises a naturally occurring BoNT/F variant of SEQ ID NO: 6, such as, *e.g.*, a BoNT/F isoform of SEQ ID NO: 6 or a BoNT/F subtype of SEQ ID NO: 6. In still another aspect of this embodiment, a BoNT/F comprises a non-naturally occurring BoNT/F variant, such as, *e.g.*, a conservative BoNT/F variant, a non-conservative BoNT/F variant or an active BoNT/F fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F comprises a non-naturally occurring BoNT/F variant of SEQ ID NO: 6, such as, *e.g.*, a conservative BoNT/F variant of SEQ ID NO: 6, a non-conservative BoNT/F variant of SEQ ID NO: 6 or an active BoNT/F fragment of SEQ ID NO: 6, or any combination thereof. In yet another aspect of this embodiment, a BoNT/F comprises a BoNT/F enzymatic domain or active fragment thereof, a BoNT/F translocation domain or active fragment thereof, a BoNT/F binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a BoNT/F comprises a BoNT/F enzymatic domain of amino acid 1-439 from SEQ ID NO: 6 or active fragment thereof, a BoNT/F translocation domain of amino acids 440-864 from SEQ ID NO: 6 or active fragment thereof, a BoNT/F binding domain of amino acids 865-1274 from SEQ ID NO: 6 or active fragment thereof, and any combination thereof.

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[065] In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 6, at least 75% amino acid identity with the SEQ ID NO: 6, at least 80% amino acid identity with SEQ ID NO: 6, at least 85% amino acid identity with SEQ ID NO: 6, at least 90% amino acid identity with SEQ ID NO: 6 or at least 95% amino acid identity with SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 6, at most 75% amino acid identity with the SEQ ID NO: 6, at most 80% amino acid identity with SEQ ID NO: 6, at most 85% amino acid identity with SEQ ID NO: 6, at most 90% amino acid identity with SEQ ID NO: 6 or at most 95% amino acid identity with SEQ ID NO: 6.

[066] In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 6.

[067] In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 6. In yet other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 6. In other aspects of this embodiment, a BoNT/F comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six,

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seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 6.

[068] In another embodiment, a Clostridial toxin comprises a BoNT/G. In an aspect of this embodiment, a BoNT/G comprises a BoNT/G enzymatic domain, a BoNT/G translocation domain and a BoNT/G binding domain. In another aspect of this embodiment, a BoNT/G comprises SEQ ID NO: 7. In another aspect of this embodiment, a BoNT/G comprises a naturally occurring BoNT/G variant, such as, *e.g.*, a BoNT/G isoform or a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G comprises a naturally occurring BoNT/G variant of SEQ ID NO: 7, such as, *e.g.*, a BoNT/G isoform of SEQ ID NO: 7 or a BoNT/G subtype of SEQ ID NO: 7. In still another aspect of this embodiment, a BoNT/G comprises a non-naturally occurring BoNT/G variant, such as, *e.g.*, a conservative BoNT/G variant, a non-conservative BoNT/G variant or an active BoNT/G fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D comprises a non-naturally occurring BoNT/G variant of SEQ ID NO: 7, such as, *e.g.*, a conservative BoNT/G variant of SEQ ID NO: 7, a non-conservative BoNT/G variant of SEQ ID NO: 7 or an active BoNT/G fragment of SEQ ID NO: 7, or any combination thereof. In yet another aspect of this embodiment, a BoNT/G comprises a BoNT/G enzymatic domain or an active fragment thereof, a BoNT/G translocation domain or an active fragment thereof, a BoNT/G binding domain or an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/G comprising a BoNT/G enzymatic domain of amino acids 1-446 from SEQ ID NO: 7 or an active fragment thereof, a BoNT/G translocation domain of amino acids 447-863 from SEQ ID NO: 7 or an active fragment thereof, a BoNT/G binding domain of amino acids 864-1297 from SEQ ID NO: 7 or an active fragment thereof, and any combination thereof.

[069] In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 7, at least 75% amino acid identity with the SEQ ID NO: 7, at least 80% amino acid identity with SEQ ID NO: 7, at least 85% amino acid identity with SEQ ID NO: 7, at least 90% amino acid identity with SEQ ID NO: 7 or at least 95% amino acid identity with SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 7, at most 75% amino acid identity with the SEQ ID NO: 7, at most 80% amino acid identity with SEQ ID NO: 7, at most 85% amino acid identity with SEQ ID NO: 7, at most 90% amino acid identity with SEQ ID NO: 7 or at most 95% amino acid identity with SEQ ID NO: 7.

[070] In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions

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relative to SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 7. In still other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 7.

[071] In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 7. In yet other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 7. In still other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 7. In other aspects of this embodiment, a BoNT/G comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 7.

[072] In another embodiment, a Clostridial toxin comprises a TeNT. In an aspect of this embodiment, a TeNT comprises a TeNT enzymatic domain, a TeNT translocation domain and a TeNT binding domain. In an aspect of this embodiment, a TeNT comprises SEQ ID NO: 8. In another aspect of this embodiment, a TeNT comprises a naturally occurring TeNT variant, such as, *e.g.*, a TeNT isoform or a TeNT subtype. In another aspect of this embodiment, a TeNT comprises a naturally occurring TeNT variant of SEQ ID NO: 8, such as, *e.g.*, a TeNT isoform of SEQ ID NO: 8 or a TeNT subtype of SEQ ID NO: 8. In still another aspect of this embodiment, a TeNT comprises a non-naturally occurring TeNT variant, such as, *e.g.*, a conservative TeNT variant, a non-conservative TeNT variant or an active TeNT fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT comprises a non-naturally occurring TeNT variant of SEQ ID NO: 8, such as, *e.g.*, a conservative TeNT variant of SEQ ID NO: 8, a non-conservative TeNT variant of SEQ ID NO: 8 or an active TeNT fragment of SEQ ID NO: 8, or any combination thereof. In yet another aspect of this embodiment, a TeNT comprising a TeNT enzymatic domain or an active fragment thereof, a TeNT translocation domain or active fragment thereof, a TeNT binding domain or active fragment thereof, and any combination thereof. In yet another aspect of

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this embodiment, a TeNT comprising a TeNT enzymatic domain of amino acids 1-457 from SEQ ID NO: 8 or active fragment thereof, a TeNT translocation domain of amino acids 458-879 from SEQ ID NO: 8 or active fragment thereof, a TeNT binding domain of amino acids 880-1315 from SEQ ID NO: 8 or active fragment thereof, and any combination thereof.

[073] In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 8, at least 75% amino acid identity with the SEQ ID NO: 8, at least 80% amino acid identity with SEQ ID NO: 8, at least 85% amino acid identity with SEQ ID NO: 8, at least 90% amino acid identity with SEQ ID NO: 8 or at least 95% amino acid identity with SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 8, at most 75% amino acid identity with the SEQ ID NO: 8, at most 80% amino acid identity with SEQ ID NO: 8, at most 85% amino acid identity with SEQ ID NO: 8, at most 90% amino acid identity with SEQ ID NO: 8 or at most 95% amino acid identity with SEQ ID NO: 8.

[074] In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 8. In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 8. In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 8. In still other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 8. In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 8.

[075] In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 8. In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 8. In yet other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 8. In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid

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deletions relative to SEQ ID NO: 8. In still other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 8. In other aspects of this embodiment, a TeNT comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 8.

[076] Aspects of the present invention provide, in part, a PAR ligand domain. As used herein, the term "PAR ligand domain" is synonymous with "tethered ligand" and "activating peptide" and means any polypeptide that can selectively bind to the PAR ligand binding domain and initiate the overall internalization mechanism whereby an activated PAR is internalized into a cell. As used herein, the term "selectively" means having a unique effect or influence or reacting in only one way or with only one thing. As used herein, the term "selectively bind" means that a PAR ligand domain is able to bind its target PAR ligand binding domain under physiological conditions, or in vitro conditions substantially approximating physiological conditions, to a statistically significantly greater degree (*i.e.*, has a smaller K_d or dissociation constant) than to other, non-target ligand binding domains. " K_d " is the molar concentration of the PAR ligand domain at which half the PAR ligand binding domains are bound by the PAR ligand domain. Thus, there is a discriminatory binding of the PAR ligand domain to the indicated target binding site.

[077] Most G protein-coupled receptors (GPCRs) are reversibly activated upon ligand binding. However, activation of protease-activated G protein-coupled receptors (PARs) occurs through an irreversible proteolytic event that results in the generation of a tethered ligand that cannot diffuse away. In essence, PARs are receptors that carry their own ligands, which remain unbound until unmasked by site-specific receptor cleavage. The coagulant protease Thrombin is the physiological activator of PAR1, PAR3 and PAR4; however, other proteases can cleave these receptors and may contribute to their function in vivo (Table 2). PAR2 is activated by multiple Trypsin-like serine proteases including Trypsin, Tryptase and coagulation proteases upstream of Thrombin, Factors VIIa and Xa, but not by Thrombin (Table 2).

[078] Currently four subtypes of human PARs are described and designated PAR1 (SEQ ID NO: 9), PAR2 (SEQ ID NO: 10), PAR3 (SEQ ID NO: 11) and PAR4 (SEQ ID NO: 12). In addition, PAR1, PAR2, PAR3 and PAR4 orthologs which exhibit at least 70% amino acid identity and at least 80% amino acid similarity have been identified in other mammals, such as, *e.g.*, the chimpanzee *Pan troglodytes*, the hamadryas baboon *Papio hamadryas*, the dog *Canis familiaris*, the mouse *Mus musculus*, the rat *Rattus norvegicus* and the chicken *Gallus gallus*. The protease cleavage site, which upon cleavage unmasks the tethered ligand, is known for all four receptors (Table 2). In human PARs, cleavage of PAR1 at R41-S42 exposes a new amino terminus ending in the hexapeptide SFLLRN, cleavage of PAR2 at R34-S35 exposes a new amino terminus ending in the hexapeptide SLIGKV, cleavage of PAR3 at K38-T39 exposes a new amino terminus ending in the hexapeptide TFRGAP, where as, cleavage of PAR4 at R47-G48 exposes a new amino terminus ending in the hexapeptide GYPGQV. A hirudin-like site distal to the

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protease cleavage site has been described in PAR1 and PAR3. This charged domain appears to help mediate the binding of Thrombin to PAR1, thereby facilitating cleavage of the protease cleavage site.

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Table 2 Summary of the Human PAR Family				
	PAR1	PAR2	PAR3	PAR4
Endogenous Activating proteases	APC Factor Xa Thrombin Trypsins	Acrosien Factor Xa Factor VIIa MT-SP1 Proteinase-3 Trypsins Trypsases	Thrombin	Cathepsin G Factor Xa Factor VIIa Plasmin Thrombin Trypsins
Exogenous Activating proteases	Granzyme A Gingipains-R	Der P1 Der P3 Der P9 Gingipains-R		Gingipains-R
Inactivating proteases	Cathepsin G Elastase Plasmin Proteinase-3 Trypsins	Cathepsin G Elastase	Cathepsin G	
Cleavage site	LDPR ⁴¹ *S ⁴² FLLRN	SKGR ³⁴ *S ³⁵ LIGKV	LPIK ³⁸ *T ³⁹ FRGAP	PAPR ⁴⁷ *G ⁴⁸ YPGQV
Localization	platelets endothelium epithelium fibroblasts myocytes neurons astrocytes	epithelium endothelium fibroblasts myocytes neurons astrocytes	platelets endothelium myocytes astrocytes	platelets endothelium myocytes astrocytes
An asterisks (*) indicates the peptide bond that is cleaved by an activating PAR protease.				

[079] Synthetic peptides representing the newly formed amino terminus tethered ligand of PARs can act as agonists for the receptor without the need for proteolysis and can initiate many of the same signaling responses elicited by proteolytically activated PARs (Table 3), see *e.g.*, Shaun R. Coughlin and Mark Kahn, *Modulation of Platelet Activation*, PCT Patent Publication WO 01/07072 (Feb. 1, 2001); Shaun R. Coughlin and Tatjana R. Faruqi, *Peptides Modulating Protease Activated Receptors and Methods of Using Same*, PCT Patent Publication WO 01/94411 (Dec. 13, 2001); Scott R. MacFarlane *et al.*, *Protease-Activated Receptors*, 53(2) Pharmacol. Rev. 245-282 (2001); and Robert M. Scarborough, *Protease-Activated Receptor-2 Antagonists and Agonists*, 1(1) Curr. Med. Chem. Cardiovasc. Hematol. Agents 73-82 (2003). Referred to as activating peptides (AP), these peptides evoke the ligand binding, the signal transduction and the signal termination steps described above. The first described AP was the 14-residue peptide SFLLRNPNDKYEPF comprising amino acids 42-55 of SEQ ID NO: 13 that behaves as an agonist for PAR1. Subsequent work has shown that not only the hexapeptide SFLLRN, but a wide range of variants were also effective, if not fully functional to elicit a cellular response (Table 3). Analysis of PAR APs using alanine scanning and site-directed mutagenesis has identified residues critical for function. For example, the residues F2, L4 and R5 are functionally important for the PAR1 AP hexapeptide SFLLRN, but substitutions of residues at other positions can be tolerated. Similar testing of the PAR2 AP hexapeptide SLIGKV indicates that L2 and R5 are essential for PAR2 AP activity whereas substitution of G4 or L6 has only a slight effect on PAR2 activation. Replacing S1 or I3 with alanine also

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reduces activity. While many PAR4 variants are functional (Table 3), the specificity of PAR4 AP requires Y2, since replacement with F generates an agonist of both PAR1 and PAR4.

Table 3. PAR Binding Domains		
PAR1	Amino Acid Sequence	SEQ ID NO:
Reference	SFLLRN	13
Variants	SFLLRN	14
	SFFLKN	133
	TFLLRN	15
	GFPGKF	16
	GYPKF	17
	GYPLKF	18
	GYPIKF	19
	G(F)PGKF	20
	GYP(Cha)KF	21
	S(F)(Cha)(Cha)RK	22
	S(F)(Cha)(Cha)(homoR)K	23
PAR2	Amino Acid Sequence	SEQ ID NO:
Reference	SLIGKV	24
Variants	SLIGRL	25
PAR3	Amino Acid Sequence	SEQ ID NO:
Reference	TFRGAP	26
Variants	SFNGGP	27
	SFNGNE	134
PAR4	Amino Acid Sequence	SEQ ID NO:
Reference	GYPGQV	28
Variants	AYPGKF	29
	TYPGKF	30
	GYPGKY	31
	GYPGKW	32
	GYPGKK	33
	GYPGKF	34
	GYPGRF	35
	GYPGFK	36
	GYPKF	37
	GFPGKF	38
	GFPGKP	39
	SYPGKF	40
	SYPKF	41
	SYPGRF	42
	SYAGKF	43
	SFPGQP	135
	SFPGQA	160
	GYPG(Orn)F	44
	G(F)PGKF	45
	GYPG(homoR)F	46
	SYPG(homoR)F	47
(Cha), cyclohexylalanine; (homoR), homoarginine; (Orn), ornithine; (F), parafluoro-phenylalanine; other letters represent the single letter amino acid code.		

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[080] It is envisioned that any and all PAR ligand domains capable of binding an inactivated PAR and eliciting the internalization of the modified Clostridial toxin-PAR complex into a cell can be useful in aspects of the present invention. It is envisioned that a PAR ligand domain of any and all lengths can be useful in aspects of the present invention with the proviso that the PAR ligand domain is capable of binding an inactivated PAR and eliciting the internalization of the modified Clostridial toxin-PAR complex into a cell. Thus, in aspects of this embodiment, a PAR ligand domain can be, *e.g.*, at least 6 amino acids in length, at least 7 amino acids in length, at least 8 amino acids in length, at least 9 amino acids in length, at least 10 amino acids in length, at least 15 amino acids in length, at least 20 amino acids in length, at least 25 amino acids in length, at least 30 amino acids in length, at least 40 amino acids in length, at least 50 amino acids in length or at least 60 amino acids in length. In other aspects of this embodiment, a PAR ligand domain can be, *e.g.*, at most 6 amino acids in length, at most 7 amino acids in length, at most 8 amino acids in length, at most 9 amino acids in length, at most 10 amino acids in length, at most 15 amino acids in length, at most 20 amino acids in length, at most 25 amino acids in length, at most 30 amino acids in length, at most 40 amino acids in length, at most 50 amino acids in length or at most 60 amino acids in length. As a non-limiting example, a PAR 1 ligand domain can comprise amino acids 1-64 of SEQ ID NO: 9, amino acids 1-55 of SEQ ID NO: 9, amino acids 1-47 of SEQ ID NO: 9, amino acids 29-64 of SEQ ID NO: 9, amino acids 42-55 of SEQ ID NO: 9 or amino acids 42-47 of SEQ ID NO: 9. As another non-limiting example, a PAR 2 ligand domain can comprise amino acids 1-59 of SEQ ID NO: 10, comprise amino acids 1-48 of SEQ ID NO: 10, comprise amino acids 1-40 of SEQ ID NO: 10, amino acids 24-59 of SEQ ID NO: 10, amino acids 35-48 of SEQ ID NO: 10 or amino acids 35-40 of SEQ ID NO: 10. As still another non-limiting example, a PAR 3 ligand domain can comprise amino acids 1-60 of SEQ ID NO: 11, comprise amino acids 1-52 of SEQ ID NO: 11, comprise amino acids 1-44 of SEQ ID NO: 11, amino acids 26-60 of SEQ ID NO: 11, amino acids 39-52 of SEQ ID NO: 11 or amino acids 39-44 of SEQ ID NO: 11. As yet another non-limiting example, a PAR 4 ligand domain can comprise amino acids 1-70 of SEQ ID NO: 12, comprise amino acids 1-61 of SEQ ID NO: 12, comprise amino acids 1-53 of SEQ ID NO: 12, amino acids 35-70 of SEQ ID NO: 12, amino acids 48-61 of SEQ ID NO: 12 or amino acids 48-53 of SEQ ID NO: 12.

[081] A PAR ligand domain useful in aspects of the invention includes, without limitation, naturally occurring PAR ligand domains, such as, *e.g.*, a PAR1 tethered ligand, a PAR2 tethered ligand, a PAR3 tethered ligand or a PAR4 tethered ligand; naturally occurring PAR ligand domain variants; and non-naturally-occurring PAR ligand domain variants, such as, *e.g.*, conservative PAR ligand domain variants, non-conservative PAR ligand domain variants and PAR ligand domain peptidomimetics. As used herein, the term "PAR ligand domain variant," whether naturally-occurring or non-naturally-occurring, means a PAR ligand domain that has at least one amino acid change from the corresponding region of the disclosed reference sequences and can be described in percent identity to the corresponding region of that reference sequence (Table 3). Any of a variety of sequence alignment methods can be used to determine percent identity, including, without limitation, global methods, local methods and hybrid

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methods, such as, *e.g.*, segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[082] As used herein, the term “naturally occurring PAR ligand domain variant” means any PAR ligand domain produced without the aid of any human manipulation, including, without limitation, PAR ligand domain isoforms produced from alternatively-spliced transcripts, PAR ligand domain isoforms produced by spontaneous mutation and PAR ligand domain subtypes.

[083] As used herein, the term “non-naturally occurring PAR ligand domain variant” means any PAR ligand domain produced with the aid of human manipulation, including, without limitation, PAR ligand domain variants produced by genetic engineering using random mutagenesis or rational design and PAR ligand domain variants produced by chemical synthesis. Non-limiting examples of non-naturally occurring PAR ligand domain variant include, *e.g.*, conservative PAR ligand domain variants, non-conservative PAR ligand domain variants and PAR ligand domain peptidomimetics.

[084] As used herein, the term “conservative PAR ligand domain variant” means a PAR ligand domain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference PAR ligand domain sequence (Table 3). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative PAR ligand domain variant can function in substantially the same manner as the reference PAR ligand domain on which the conservative PAR ligand domain variant is based, and can be substituted for the reference PAR ligand domain in any aspect of the present invention. A conservative PAR ligand domain variant may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids or five or more amino acids from the reference PAR ligand domain on which the conservative PAR ligand domain variant is based. A conservative PAR ligand domain variant can also possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference PAR ligand domain on which the conservative PAR ligand domain variant is based. Non-limiting examples of a conservative PAR ligand domain variant include, *e.g.*, conservative PAR1 ligand domain variants, conservative PAR2 ligand domain variants, conservative PAR3 ligand domain variants and conservative PAR4 ligand domain variants.

[085] As used herein, the term “non-conservative PAR ligand domain variant” means a PAR ligand domain in which 1) at least one amino acid is deleted from the reference PAR ligand domain on which the non-conservative PAR ligand domain variant is based; 2) at least one amino acid added to the reference PAR ligand domain on which the non-conservative PAR ligand domain is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference PAR ligand domain sequence (Table 3). A

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non-conservative PAR ligand domain variant can function in substantially the same manner as the reference PAR ligand domain on which the non-conservative PAR ligand domain is based, and can be substituted for the reference PAR ligand domain in any aspect of the present invention. A non-conservative PAR ligand domain variant can add one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids, five or more amino acids, and ten or more amino acids to the reference PAR ligand domain on which the non-conservative PAR ligand domain variant is based. A non-conservative PAR ligand domain may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids or five or more amino acids from the reference PAR ligand domain on which the non-conservative PAR ligand domain variant is based. A non-conservative PAR ligand domain variant can also possess at least 50% amino acid identity, 65% amino acid identity, 75% amino acid identity, 85% amino acid identity or 95% amino acid identity to the reference PAR ligand domain on which the non-conservative PAR ligand domain variant is based. Non-limiting examples of a non-conservative PAR ligand domain variant include, *e.g.*, non-conservative PAR1 ligand domain variants, non-conservative PAR2 ligand domain variants, non-conservative PAR3 ligand domain variants and non-conservative PAR4 ligand domain variants.

[086] As used herein, the term "PAR ligand domain peptidomimetic" means a PAR ligand domain that has at least one amino acid substituted by a non-natural oligomer that has at least one property similar to that of the first amino acid. Examples of properties include, without limitation, topography of a peptide primary structural element, functionality of a peptide primary structural element, topology of a peptide secondary structural element, functionality of a peptide secondary structural element, of the like, or any combination thereof. A PAR ligand domain peptidomimetic can function in substantially the same manner as the reference PAR ligand domain on which the PAR ligand domain peptidomimetic is based, and can be substituted for the reference PAR ligand domain in any aspect of the present invention. A PAR ligand domain peptidomimetic may substitute one or more amino acids, two or more amino acids, three or more amino acids, four or more amino acids or five or more amino acids from the reference PAR ligand domain on which the PAR ligand domain peptidomimetic is based. A PAR ligand domain peptidomimetic can also possess at least 50% amino acid identity, at least 65% amino acid identity, at least 75% amino acid identity, at least 85% amino acid identity or at least 95% amino acid identity to the reference PAR ligand domain on which the PAR ligand domain peptidomimetic is based. For examples of peptidomimetic methods see, *e.g.*, Amy S. Ripka & Daniel H. Rich, Peptidomimetic design, 2(4) CURR. OPIN. CHEM. BIOL. 441-452 (1998); and M. Angels Estiarte & Daniel H. Rich, *Peptidomimetics for Drug Design*, 803-861 (BURGER'S MEDICINAL CHEMISTRY AND DRUG DISCOVERY Vol. 1 PRINCIPLE AND PRACTICE, Donald J. Abraham ed., Wiley-Interscience, 6th ed 2003). Non-limiting examples of a PAR ligand domain peptidomimetic include, *e.g.*, PAR1 ligand domain peptidomimetics, PAR2 ligand domain peptidomimetics, PAR3 ligand domain peptidomimetics and PAR4 ligand domain peptidomimetics.

[087] Thus, in an embodiment, a PAR ligand domain comprises a naturally occurring PAR ligand domain variant, such as, *e.g.*, a PAR ligand domain isoform or a PAR ligand domain subtype. In another

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embodiment a PAR ligand domain comprises a non-naturally occurring PAR ligand domain variant, such as, *e.g.*, a conservative PAR ligand domain variant, a non-conservative PAR ligand domain variant or a PAR ligand domain peptidomimetic, or any combination thereof.

[088] In another embodiment, a PAR ligand domain comprises a PAR1 ligand domain. In an aspect of this embodiment, a PAR1 ligand domain comprises SEQ ID NO: 13. In another aspect of this embodiment, a PAR1 ligand domain comprises a naturally occurring PAR1 ligand domain variant, such as, *e.g.*, a PAR1 ligand domain isoform or a PAR1 ligand domain subtype. In another aspect of this embodiment, a PAR1 ligand domain comprises a naturally occurring PAR1 ligand domain variant of SEQ ID NO: 13, such as, *e.g.*, a PAR1 ligand domain isoform of SEQ ID NO: 13 or a PAR1 ligand domain subtype of SEQ ID NO: 13. In still another aspect of this embodiment, a PAR1 ligand domain comprises a non-naturally occurring PAR1 ligand domain variant, such as, *e.g.*, a conservative PAR1 ligand domain variant, a non-conservative PAR1 ligand domain variant or a PAR1 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a PAR1 ligand domain comprises a non-naturally occurring PAR1 ligand domain variant of SEQ ID NO: 13, such as, *e.g.*, a conservative PAR1 ligand domain variant of SEQ ID NO: 13, a non-conservative PAR1 ligand domain variant of SEQ ID NO: 13 or a PAR1 ligand domain peptidomimetic of SEQ ID NO: 13, or any combination thereof. In other aspects of this embodiment, a PAR1 ligand domain comprises SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 or SEQ ID NO: 133.

[089] In other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 13, at least 67% amino acid identity with the SEQ ID NO: 13, or at least 83% amino acid identity with SEQ ID NO: 13. In still other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 13, at most 67% amino acid identity with the SEQ ID NO: 13, at most 83% amino acid identity with SEQ ID NO: 13.

[090] In other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ

ID NO: 13. In still other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 13.

[091] In other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at most two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at least two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a PAR1 ligand domain comprises a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 13.

[092] In another embodiment, a PAR ligand domain comprises a PAR2 ligand domain. In an aspect of this embodiment, a PAR2 ligand domain comprises SEQ ID NO: 24. In another aspect of this embodiment, a PAR2 ligand domain comprises a naturally occurring PAR2 ligand domain variant, such as, *e.g.*, a PAR2 ligand domain isoform or a PAR2 ligand domain subtype. In another aspect of this embodiment, a PAR2 ligand domain comprises a naturally occurring PAR2 ligand domain variant of SEQ ID NO: 24, such as, *e.g.*, a PAR2 ligand domain isoform of SEQ ID NO: 24 or a PAR2 ligand domain subtype of SEQ ID NO: 24. In still another aspect of this embodiment, a PAR2 ligand domain comprises a non-naturally occurring PAR2 ligand domain variant, such as, *e.g.*, a conservative PAR2 ligand domain variant, a non-conservative PAR2 ligand domain variant or a PAR2 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a PAR2 ligand domain comprises a non-naturally occurring PAR2 ligand domain variant of SEQ ID NO: 24, such as, *e.g.*, a conservative PAR2 ligand domain variant of SEQ ID NO: 24, a non-conservative PAR2 ligand domain variant of SEQ ID NO: 24 or a PAR2 ligand domain peptidomimetic of SEQ ID NO: 24, or any combination thereof. In other aspects of this embodiment, a PAR2 ligand domain comprises SEQ ID NO: 24 or SEQ ID NO: 25.

[093] In other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 24, at least 67% amino acid identity with the SEQ ID NO: 24, or at least 83% amino acid identity with SEQ ID NO: 24. In still other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 24, at most 67% amino acid identity with the SEQ ID NO: 24, at most 83% amino acid identity with SEQ ID NO: 24.

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[094] In other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 24.

[095] In other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at least two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a PAR2 ligand domain comprises a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 24.

[096] In another embodiment, a PAR ligand domain comprises a PAR3 ligand domain. In an aspect of this embodiment, a PAR3 ligand domain comprises SEQ ID NO: 26. In another aspect of this embodiment, a PAR3 ligand domain comprises a naturally occurring PAR3 ligand domain variant, such as, *e.g.*, a PAR3 ligand domain isoform or a PAR3 ligand domain subtype. In another aspect of this embodiment, a PAR3 ligand domain comprises a naturally occurring PAR3 ligand domain variant of SEQ ID NO: 26, such as, *e.g.*, a PAR3 ligand domain isoform of SEQ ID NO: 26 or a PAR3 ligand domain subtype of SEQ ID NO: 26. In still another aspect of this embodiment, a PAR3 ligand domain comprises a non-naturally occurring PAR3 ligand domain variant, such as, *e.g.*, a conservative PAR3 ligand domain variant, a non-conservative PAR3 ligand domain variant or a PAR3 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a PAR3 ligand domain comprises a non-naturally occurring PAR3 ligand domain variant of SEQ ID NO: 26, such as, *e.g.*, a conservative PAR3 ligand domain variant of SEQ ID NO: 26, a non-conservative PAR3 ligand domain variant of SEQ ID NO: 26 or a PAR3 ligand domain peptidomimetic of SEQ ID NO: 26, or any combination thereof. In other

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aspects of this embodiment, a PAR3 ligand domain comprises SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 134.

[097] In other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 26, at least 67% amino acid identity with the SEQ ID NO: 26, or at least 83% amino acid identity with SEQ ID NO: 26. In still other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 26, at most 67% amino acid identity with the SEQ ID NO: 26, at most 83% amino acid identity with SEQ ID NO: 26.

[098] In other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 26.

[099] In other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at least two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a PAR3 ligand domain comprises a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 26.

[0100] In another embodiment, a PAR ligand domain comprises a PAR4 ligand domain. In an aspect of this embodiment, a PAR4 ligand domain comprises SEQ ID NO: 28. In another aspect of this embodiment, a PAR4 ligand domain comprises a naturally occurring PAR4 ligand domain variant, such

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as, *e.g.*, a PAR4 ligand domain isoform or a PAR4 ligand domain subtype. In another aspect of this embodiment, a PAR4 ligand domain comprises a naturally occurring PAR4 ligand domain variant of SEQ ID NO: 28, such as, *e.g.*, a PAR4 ligand domain isoform of SEQ ID NO: 28 or a PAR4 ligand domain subtype of SEQ ID NO: 28. In still another aspect of this embodiment, a PAR4 ligand domain comprises a non-naturally occurring PAR4 ligand domain variant, such as, *e.g.*, a conservative PAR4 ligand domain variant, a non-conservative PAR4 ligand domain variant or a PAR4 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a PAR4 ligand domain comprises a non-naturally occurring PAR4 ligand domain variant of SEQ ID NO: 28, such as, *e.g.*, a conservative PAR4 ligand domain variant of SEQ ID NO: 28, a non-conservative PAR4 ligand domain variant of SEQ ID NO: 28 or a PAR4 ligand domain peptidomimetic of SEQ ID NO: 28, or any combination thereof. In other aspects of this embodiment, a PAR4 ligand domain comprises SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 135 or SEQ ID NO: 160.

[0101] In other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 28, at least 67% amino acid identity with the SEQ ID NO: 28, or at least 83% amino acid identity with SEQ ID NO: 28. In still other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 28, at most 67% amino acid identity with the SEQ ID NO: 28, at most 83% amino acid identity with SEQ ID NO: 28.

[0102] In other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 28.

[0103] In other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at most two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at least two,

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three or four contiguous amino acid substitutions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a PAR4 ligand domain comprises a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 28.

[0104] When a PAR protease cleaves the extracellular amino-terminus of a PAR, a new amino acid terminus is generated that functions as a tethered ligand. Currently it is believed that the amino terminus location of the tethered ligand is critical for the ligand to effectively bind to the second extracellular loop region of the receptor that comprises the ligand binding domain. It is envisioned that a modified Clostridial toxin of the present specification can comprise a PAR ligand domain in any and all locations with the proviso that formation of the di-chain molecule will result in the free amino terminus of the PAR ligand domain. Non-limiting examples include, locating the PAR ligand domain at the amino terminus of the Clostridial toxin enzymatic domain; locating the PAR ligand domain at the amino terminus of the Clostridial toxin translocation domain; and locating the PAR ligand domain at the amino terminus of the Clostridial toxin binding domain (FIG. 4).

[0105] Thus, in an embodiment, a modified Clostridial toxin comprises a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin enzymatic domain. In an aspect of this embodiment, the PAR ligand domain can be located at the amino terminus of the enzymatic domain when the amino to carboxyl linear organization of the Clostridial toxin single chain molecule is enzymatic domain, translocation domain and binding domain. In another aspect of this embodiment, the PAR ligand domain can be located at the amino terminus of the enzymatic domain when the amino to carboxyl linear organization of the Clostridial toxin single chain molecule is enzymatic domain, binding domain and translocation domain.

[0106] In another embodiment, a modified Clostridial toxin comprises a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin translocation domain. In an aspect of this embodiment, the PAR ligand domain can also be located at the amino terminus of the translocation domain when the amino to carboxyl linear organization of the Clostridial toxin single chain molecule is binding domain, enzymatic domain and translocation domain. In another aspect of this embodiment, the PAR ligand domain can also be located at the amino terminus of the translocation domain when the amino to carboxyl linear organization of the Clostridial toxin single chain

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molecule is enzymatic domain, translocation domain and binding domain.

[0107] In still another embodiment, a modified Clostridial toxin comprises a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; wherein the PAR ligand domain is located at the amino terminus of the Clostridial toxin binding domain. In an aspect of this embodiment, the PAR ligand domain can also be located at the amino terminus of the binding domain when the amino to carboxyl linear organization of the Clostridial toxin single chain molecule is enzymatic domain, binding domain and translocation domain.

Table 4: Amino Terminus Region			
Toxin	SEQ ID NO	PAR Ligand Domain	Light Chain Region
BoNT/A	1	M-PAR Ligand Domain	PFVNKQFNYKDPVNGVDIA
BoNT/B	2	M-PAR Ligand Domain	PVTINNFNYNDPIDNNNII
BoNT/C1	3	M-PAR Ligand Domain	PITINNFNYSDPVDNKNIL
BoNT/D	4	M-PAR Ligand Domain	TWPVKDFNYSDPVNDNDIL
BoNT/E	5	M-PAR Ligand Domain	PKINSFNYNDPVNDRTILY
BoNT/F	6	M-PAR Ligand Domain	PVAINSFNYNDPVNDDTIL
BoNT/G	7	M-PAR Ligand Domain	PVNIKXFNYNDPINDDII
TeNT	8	M-PAR Ligand Domain	PITINNFYSDPVNNDTII
The amino acid sequence displayed are as follows: BoNT/A, residues 2-20 of SEQ ID No: 1; BoNT/B, residues 2-20 of SEQ ID No: 2; BoNT/C1, residues v of SEQ ID No: 3; BoNT/D, residues 2-20 of SEQ ID No: 4; BoNT/E, residues 2-20 of SEQ ID No: 5; BoNT/F, residues 2-20 of SEQ ID No: 6; BoNT/G, residues 2-20 of SEQ ID No: 7; and TeNT, residues 2-20 of SEQ ID No: 8.			

[0108] In yet another embodiment, the location of the PAR ligand domain is located at the amino terminus of the modified Clostridial toxin. In such a location, the PAR ligand domain can bind to a ligand binding domain of a PAR; proteolytic cleavage is not necessary to unmask the PAR ligand domain. As used herein, the term "unmask" means that the amino terminus of a PAR ligand domain is free to bind to a ligand binding domain of a PAR. It is known in the art that when adding a polypeptide that is operationally-linked to the amino terminus of another polypeptide comprising the start methionine that this methionine residue can be deleted (Table 4). This is due to the fact that the added polypeptide will contain a new start methionine and that the original start methionine may reduce optimal expression of the fusion protein.

[0109] In yet another embodiment, the location of the PAR ligand domain is not located at the amino terminus of the modified Clostridial toxin. In such a location, the PAR ligand domain can not bind to a ligand binding domain of a PAR. The PAR ligand domain is considered masked because it is necessary to unmask a PAR ligand domain so that this domain can bind to a ligand binding domain of a PAR. As used herein, the term "masked" means that the amino terminus of a PAR ligand domain is unable to bind

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to the ligand binding domain of a PAR. To unmask a PAR ligand domain of a modified Clostridial toxin, a protease cleavage site can be placed in front of the PAR ligand domain in such a manner that, upon cleavage with an appropriate protease, the masked PAR ligand domain becomes unmasked and is now capable of binding a PAR ligand binding domain. It is envisioned that any and all proteases that can cleave a modified Clostridial toxin disclosed in the present specification so as to unmask a PAR ligand domain can be used, including without limitation, a Clostridial toxin protease cleavage site found in the di-chain loop, a PAR protease cleavage site used to unmask the tethered ligand *in vivo*, and an exogenous protease cleavage site.

[0110] As mentioned above, a Clostridial toxin is converted from a single polypeptide form into a di-chain molecule by proteolytic cleavage. While the identity of the protease is currently unknown, the di-chain loop protease cleavage site for many Clostridial toxins has been determined. In BoNTs, cleavage at K448-A449 converts the single polypeptide form of BoNT/A into the di-chain form; cleavage at K441-A442 converts the single polypeptide form of BoNT/B into the di-chain form; cleavage at K449-T450 converts the single polypeptide form of BoNT/C1 into the di-chain form; cleavage at R445-D446 converts the single polypeptide form of BoNT/D into the di-chain form; cleavage at R422-K423 converts the single polypeptide form of BoNT/E into the di-chain form; cleavage at K439-A440 converts the single polypeptide form of BoNT/F into the di-chain form; and cleavage at K446-S447 converts the single polypeptide form of BoNT/G into the di-chain form. Proteolytic cleavage of the single polypeptide form of TeNT at A457-S458 results in the di-chain form. Such a di-chain loop protease cleavage site is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. However, it should also be noted that additional cleavage sites within the di-chain loop also appear to be cleaved resulting in the generation of a small peptide fragment being lost. As a non-limiting example, BoNT/A single-chain polypeptide cleavage ultimately results in the loss of a ten amino acid fragment within the di-chain loop.

[0111] Thus, in an embodiment, proteolytic cleavage of an endogenous Clostridial toxin di-chain loop protease cleavage site is used to unmask a PAR ligand domain. In aspects of this embodiment, a PAR ligand domain is unmasked by proteolytic cleavage of, *e.g.*, a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site or a TeNT di-chain loop protease cleavage site.

[0112] A wide variety of endogenous PAR proteases are known to cleave a PAR in such a manner as to unmask the tethered ligand and, therefore, can also be used to unmask the PAR ligand domain. The coagulant protease Thrombin is the physiological activator of PAR1, PAR3 and PAR4. Other PAR proteases, however, can also activate PAR receptors by proteolytic cleavage including, without limitation, APC, Cathepsin G, Factor VIIa, Factor Xa, Granzyme A, Gingipains-R, Plasmin and Trypsins (Table 2). PAR2 can also be activated by multiple proteases including, without limitation, Acrosien, Der P1, Der P3,

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Der P9, Factor VIIa, Factor Xa, Gingipains-R, MT-SP1, Proteinase-3, Trypsins and Trypsins (Table 2). It is envisioned that both endogenous protease cleavage sites found associated with a particular PAR ligand domain, as well as exogenous protease cleavage sites from other PAR ligand domains can be used to cleave a modified Clostridial toxin disclosed in the present specification in order to unmask the PAR ligand binding domain. Such a PAR protease cleavage site is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. As a non-limiting example, a PAR1 ligand domain can be unmasked using the protease cleavage site associated with the *in vivo* PAR1 molecule, or a PAR1 ligand domain can be unmasked using the protease cleavage site associated with PAR2, PAR3 or PAR4 (Table 2). As another non-limiting example, a PAR2 ligand domain can be unmasked using the protease cleavage site associated with the *in vivo* PAR2 molecule, or a PAR2 ligand domain can be unmasked using the protease cleavage site associated with PAR1, PAR3 or PAR4 (Table 2). As still another non-limiting example, a PAR3 ligand domain can be unmasked using the protease cleavage site associated with the *in vivo* PAR3 molecule, or a PAR3 ligand domain can be unmasked using the protease cleavage site associated with PAR1, PAR2 or PAR4 (Table 2). As yet another non-limiting example, a PAR4 ligand domain can be unmasked using the protease cleavage site associated with the *in vivo* PAR4 molecule, or a PAR4 ligand domain can be unmasked using the protease cleavage site associated with PAR1, PAR2 or PAR3 (Table 2).

[0113] Thus, in an embodiment, proteolytic cleavage of an endogenous PAR1 protease cleavage site is used to unmask a PAR ligand domain. In aspects of this embodiment, a PAR ligand domain is unmasked by proteolytic cleavage of, *e.g.*, an APC protease cleavage site, a Factor Xa protease cleavage site, a Granzyme A protease cleavage site, a Gingipains-R protease cleavage site, a Thrombin protease cleavage site or a Trypsin protease cleavage site. In other aspects of this embodiment, a PAR1 protease cleavage site is cleaved by, *e.g.*, an APC protease, a Factor Xa protease, a Granzyme A protease, a Gingipains-R protease, a Thrombin protease or a Trypsin protease.

[0114] In another embodiment, proteolytic cleavage of an endogenous PAR2 protease cleavage site is used to unmask a PAR ligand domain. In aspects of this embodiment, a PAR ligand domain is unmasked by proteolytic cleavage of, *e.g.*, an Acrosien protease cleavage site, a Der P1 protease cleavage site, a Der P3 protease cleavage site, a Der P9 protease cleavage site, a Factor VIIa protease cleavage site, a Factor Xa protease cleavage site, a Gingipains-R protease cleavage site, a MT-SP1 protease cleavage site, a Proteinase-3 protease cleavage site, a Trypsin protease cleavage site or a Trypsin protease cleavage site. In other aspects of this embodiment, a PAR2 protease cleavage site is cleaved by, *e.g.*, an Acrosien protease, a Der P1 protease, a Der P3 protease, a Der P9 protease, a Factor VIIa protease, a Factor Xa protease, a Gingipains-R protease, a MT-SP1 protease, a Proteinase-3 protease, a Trypsin protease or a Trypsin protease.

[0115] In another embodiment, proteolytic cleavage of an endogenous PAR3 protease cleavage site is used to unmask a PAR ligand domain. In an aspect of this embodiment, a PAR ligand domain is

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unmasked by proteolytic cleavage of, *e.g.*, a Thrombin protease cleavage site. In another aspect of this embodiment, a PAR3 protease cleavage site is cleaved by, *e.g.*, a Thrombin protease.

[0116] In another embodiment, proteolytic cleavage of an endogenous PAR4 protease cleavage site is used to unmask a PAR ligand domain. In aspects of this embodiment, a PAR ligand domain is unmasked by proteolytic cleavage of, *e.g.*, a Cathepsin G protease cleavage site, a Factor VIIa protease cleavage site, a Factor Xa protease cleavage site, a Gingipains-R protease cleavage site, a Plasmin protease cleavage site, a Thrombin protease cleavage site or a Trypsin protease cleavage site. In other aspects of this embodiment, a PAR4 protease cleavage site is cleaved by, *e.g.*, a Cathepsin G protease, a Factor VIIa protease, a Factor Xa protease, a Gingipains-R protease, a Plasmin protease, a Thrombin protease or a Trypsin protease.

[0117] It is also envisioned that an exogenous protease cleavage site can be used to unmask a PAR ligand domain. Such an exogenous protease cleavage site is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. Non-limiting examples of protease cleavage sites include, *e.g.*, an enterokinase cleavage site (Table 5); a Thrombin cleavage site (Table 5); a Factor Xa cleavage site (Table 5); a human rhinovirus 3C protease cleavage site (Table 4); a tobacco etch virus (TEV) protease cleavage site (Table 5); a dipeptidyl aminopeptidase cleavage site and a small ubiquitin-like modifier (SUMO)/ubiquitin-like protein-1 (ULP-1) protease cleavage site, such as, *e.g.*, MADSEVNQEAKPEVKP EVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQKGEMDSLRFYDGIHQADQTPEDLDMEDNDI IEAHREQIGG (SEQ ID. NO: 67). As a non-limiting example, a PAR1 ligand domain can be unmasked using a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site (Table 5). As another non-limiting example, a PAR2 ligand domain can be unmasked using a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site (Table 5). As still another non-limiting example, a PAR3 ligand domain can be unmasked using a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site (Table 5). As yet another non-limiting example, a PAR4 ligand domain can be unmasked using a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site (Table 5).

[0118] Thus, in an embodiment, proteolytic cleavage of an exogenous protease cleavage site is used to unmask a PAR ligand domain. In aspects of this embodiment, a PAR ligand domain is unmasked by proteolytic cleavage of, *e.g.*, a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease

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cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site. In other aspects of this embodiment, a PAR protease cleavage site is cleaved by, *e.g.*, a bovine enterokinase protease, a Tobacco Etch Virus protease, a Human Rhinovirus 3C protease, a SUMO/ULP-1 protease, a Thrombin protease or a Factor Xa protease, thereby unmasking a PAR ligand domain.

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Table 5. Exogenous Protease Cleavage Sites			
Protease Cleavage Site	Consensus Sequence	Non-limiting Examples	SEQ ID NO
Bovine enterokinase	DDDDK*	DDDDK*	50
Tobacco Etch Virus (TEV)	E P ⁵ P ⁴ YP ² Q*(G/S), where P ² , P ⁴ and P ⁵ can be any amino acid	ENLYFQ*G	51
		ENLYFQ*S	52
		ENIYTQ*G	53
		ENIYTQ*S	54
		ENIYLQ*G	55
		ENIYLQ*S	56
		ENVYFQ*G	57
		ENVYSQ*S	58
		ENVYSQ*G	59
		ENVYSQ*S	60
Human Rhinovirus 3C	P ⁵ P ⁴ LFQ*GP where P ⁴ is G, A, V, L, I, M, S or T and P ⁵ can any amino acid, with D or E preferred.	EALFQ*GP	61
		EVLfQ*GP	62
		ELLFQ*GP	63
		DALFQ*GP	64
		DVLfQ*GP	65
		DLLFQ*GP	66
SUMO/ULP-1	Tertiary structure	polypeptide-G*	67
Thrombin	P ³ P ² (R/K)*P ¹ , where P ³ is any amino acid and P ² or P ¹ is G with the other position being any amino acid	GVR*G	68
		SAR*G	69
		SLR*G	70
		DGR*I	71
		QGK*I	72
Thrombin	P ⁴ P ³ P(R/K)*P ¹ P ² where P ¹ and P ² can be any amino acid except for acidic amino acids like D or E; and P ³ and P ⁴ are hydrophobic amino acids like F, L, I, Y, W, V, M, P, C or A	LVPR*GS	73
		LVPK*GS	74
		FIPR*TF	75
		VLPR*SF	76
		IVPR*SF	77
		IVPR*GY	78
		VVPR*GV	79
		VLPR*LI	80
		VMPR*SL	81
		MFPR*SL	82
Coagulation Factor Xa	I(E/D)GR*	IDGR*	83
		IEGR*	84
An asterisks (*) indicates the peptide bond that is cleaved by the indicated protease.			

[0119] In another embodiment, proteolytic cleavage of an exogenous protease cleavage site is used to unmask a PAR1 ligand domain. In aspects of this embodiment, a PAR1 ligand domain is unmasked by proteolytic cleavage of, *e.g.*, a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site. In other aspects of this embodiment, a PAR1 protease cleavage site is cleaved by, *e.g.*, a bovine enterokinase protease, a Tobacco Etch Virus protease, a Human Rhinovirus 3C protease, a SUMO/ULP-1 protease, a Thrombin protease or a Factor Xa protease, thereby unmasking a PAR1 ligand domain.

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[0120] In another embodiment, proteolytic cleavage of an exogenous protease cleavage site is used to unmask a PAR2 ligand domain. In aspects of this embodiment, a PAR2 ligand domain is unmasked by proteolytic cleavage of, *e.g.*, a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site. In other aspects of this embodiment, a PAR2 protease cleavage site is cleaved by, *e.g.*, a bovine enterokinase protease, a Tobacco Etch Virus protease, a Human Rhinovirus 3C protease, a SUMO/ULP-1 protease, a Thrombin protease or a Factor Xa protease, thereby unmasking a PAR2 ligand domain.

[0121] In still another embodiment, proteolytic cleavage of an exogenous protease cleavage site is used to unmask a PAR3 ligand domain. In aspects of this embodiment, a PAR3 ligand domain is unmasked by proteolytic cleavage of, *e.g.*, a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site. In other aspects of this embodiment, a PAR3 protease cleavage site is cleaved by, *e.g.*, a bovine enterokinase protease, a Tobacco Etch Virus protease, a Human Rhinovirus 3C protease, a SUMO/ULP-1 protease, a Thrombin protease or a Factor Xa protease, thereby unmasking a PAR3 ligand domain.

[0122] In another embodiment, proteolytic cleavage of an exogenous protease cleavage site is used to unmask a PAR4 ligand domain. In aspects of this embodiment, a PAR4 ligand domain is unmasked by proteolytic cleavage of, *e.g.*, a bovine enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Human Rhinovirus 3C protease cleavage site, a SUMO/ULP-1 protease cleavage site, a Thrombin protease cleavage site or a Factor Xa protease cleavage site. In other aspects of this embodiment, a PAR4 protease cleavage site is cleaved by, *e.g.*, a bovine enterokinase protease, a Tobacco Etch Virus protease, a Human Rhinovirus 3C protease, a SUMO/ULP-1 protease, a Thrombin protease or a Factor Xa protease, thereby unmasking a PAR4 ligand domain.

[0123] It is understood that a modified Clostridial toxin disclosed in the present specification can optionally include one or more additional components. As a non-limiting example of an optional component, a modified Clostridial toxin can further comprise a flexible region comprising a flexible spacer. Non-limiting examples of a flexible spacer include, *e.g.*, a G-spacer GGGGS (SEQ ID NO: 48) or an A-spacer EAAAK (SEQ ID NO: 49). A flexible region comprising flexible spacers can be used to adjust the length of a polypeptide region in order to optimize a characteristic, attribute or property of a polypeptide. Such a flexible region is operably-linked in-frame to the modified Clostridial toxin as a fusion protein. As a non-limiting example, a polypeptide region comprising one or more flexible spacers in tandem can be used to better expose a protease cleavage site thereby facilitating cleavage of that site by a protease. As another non-limiting example, a polypeptide region comprising one or more flexible spacers in tandem can be used to better present a ligand domain, thereby facilitating the binding of that ligand domain to its binding domain on a receptor.

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[0124] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise a flexible region comprising a flexible spacer. In another embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise flexible region comprising a plurality of flexible spacers in tandem. In aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at least 1 G-spacer, at least 2 G-spacers, at least 3 G-spacers, at least 4 G-spacers or at least 5 G-spacers. In other aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at most 1 G-spacer, at most 2 G-spacers, at most 3 G-spacers, at most 4 G-spacers or at most 5 G-spacers. In still other aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at least 1 A-spacer, at least 2 A-spacers, at least 3 A-spacers, at least 4 A-spacers or at least 5 A-spacers. In still other aspects of this embodiment, a flexible region can comprise in tandem, *e.g.*, at most 1 A-spacer, at most 2 A-spacers, at most 3 A-spacers, at most 4 A-spacers or at most 5 A-spacers. In another aspect of this embodiment, a modified Clostridial toxin can comprise a flexible region comprising one or more copies of the same flexible spacers, one or more copies of different flexible-spacer regions, or any combination thereof.

[0125] As another non-limiting example of an optional component, a modified Clostridial toxin can further comprise an epitope-binding region. An epitope-binding region can be used in a wide variety of procedures involving, *e.g.*, protein purification and protein visualization. Such an epitope-binding region is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. Non-limiting examples of an epitope-binding region include, *e.g.*, FLAG, Express™, human Influenza virus hemagglutinin (HA), human p62^{c-Myc} protein (c-MYC), Vesicular Stomatitis Virus Glycoprotein (VSV-G), glycoprotein-D precursor of Herpes simplex virus (HSV), V5, and AU1; affinity-binding, such as, *e.g.*, polyhistidine (HIS), streptavidin binding peptide (strep), and biotin or a biotinylation sequence; peptide-binding regions, such as, *e.g.*, the glutathione binding domain of glutathione-S-transferase, the calmodulin binding domain of the calmodulin binding protein, and the maltose binding domain of the maltose binding protein. Non-limiting examples of specific protocols for selecting, making and using an appropriate binding peptide are described in, *e.g.*, Epitope Tagging, pp. 17.90-17.93 (Sambrook and Russell, eds., Molecular Cloning A Laboratory Manual, Vol. 3, 3rd ed. 2001); Antibodies: A Laboratory Manual (Edward Harlow & David Lane, eds., Cold Spring Harbor Laboratory Press, 2nd ed. 1998); and Using Antibodies: A Laboratory Manual: Portable Protocol No. 1 (Edward Harlow & David Lane, Cold Spring Harbor Laboratory Press, 1998). In addition, non-limiting examples of binding peptides as well as well-characterized reagents, conditions and protocols are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[0126] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise an epitope-binding region. In another embodiment, a modified Clostridial toxin disclosed

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in the present specification can further comprises a plurality of epitope-binding regions. In aspects of this embodiment, a modified Clostridial toxin can comprise, *e.g.*, at least 1 epitope-binding region, at least 2 epitope-binding regions, at least 3 epitope-binding regions, at least 4 epitope-binding regions or at least 5 epitope-binding regions. In other aspects of this embodiment, a modified Clostridial toxin can comprise, *e.g.*, at most 1 epitope-binding region, at most 2 epitope-binding regions, at most 3 epitope-binding regions, at most 4 epitope-binding regions or at most 5 epitope-binding regions. In another aspect of this embodiment, a modified Clostridial toxin can comprise one or more copies of the same epitope-binding region, one or more copies of different epitope-binding regions, or any combination thereof. The location of an epitope-binding region can be in various positions, including, without limitation, at the amino terminus of a modified Clostridial toxin, within a modified Clostridial toxin, or at the carboxyl terminus of a modified Clostridial toxin.

[0127] As still another non-limiting example of an optional component, a modified Clostridial toxin can further comprise an exogenous protease cleavage site. An exogenous protease cleavage site can be used in a wide variety of procedures involving, *e.g.*, removal of an epitope-binding region by proteolytic cleavage, conversion of a Clostridial toxin single chain polypeptide into the di-chain form or, as mentioned above, unmasking of a PAR ligand domain. Such an exogenous protease cleavage site is operably-linked in-frame to a modified Clostridial toxin as a fusion protein. Non-limiting examples of protease cleavage sites include, *e.g.*, an enterokinase cleavage site (Table 5); a Thrombin cleavage site (Table 5); a Factor Xa cleavage site (Table 5); a human rhinovirus 3C protease cleavage site (Table 4); a tobacco etch virus (TEV) protease cleavage site (Table 5); a dipeptidyl aminopeptidase cleavage site and a small ubiquitin-like modifier (SUMO)/ubiquitin-like protein-1(ULP-1) protease cleavage site, such as, *e.g.*, MADSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFKRQ GKEMDSL RFLY DGIRIQADQTPEDLDMEDNDIIEAHREQIGG (SEQ ID. NO: 67).

[0128] Thus, in an embodiment, a modified Clostridial toxin disclosed in the present specification can further comprise an exogenous protease cleavage site. In another embodiment, a modified Clostridial toxin disclosed in the present specification can further comprises a plurality of exogenous protease cleavage sites. In aspects of this embodiment, a modified Clostridial toxin can comprise, *e.g.*, at least 1 exogenous protease cleavage site, at least 2 exogenous protease cleavage sites, at least 3 exogenous protease cleavage sites, at least 4 exogenous protease cleavage sites or at least 5 exogenous protease cleavage sites. In other aspects of this embodiment, a modified Clostridial toxin can comprise, *e.g.*, at most 1 exogenous protease cleavage site, at most 2 exogenous protease cleavage sites, at most 3 exogenous protease cleavage sites, at most 4 exogenous protease cleavage sites or at most 5 exogenous protease cleavage sites. In another aspect of this embodiment, a modified Clostridial toxin can comprise one or more copies of the same exogenous protease cleavage site, one or more copies of different exogenous protease cleavage sites, or any combination thereof.

[0129] The location of an exogenous protease cleavage site may be in a variety of positions, including, without limitation, between an epitope-binding region and a modified Clostridial toxin in order to facilitate removal of the epitope-binding region by proteolytic cleavage or within the di-chain loop of the modified Clostridial toxin in order to facilitate the conversion of the single-chain polypeptide form of the toxin into the di-chain form.

[0130] It is envisioned that an exogenous protease cleavage site can be used to remove an epitope-binding region. As mentioned above, epitope binding regions can be used in protein purification procedures and it is often desirable to remove such epitope binding regions after the protein is purified. A common way of doing so is to have a protease cleavage site in between the protein of interest and the epitope binding region, whereby proteolytic cleavage of the protease cleavage site separates the protein of interest from the epitope binding region. Non-limiting examples of protease cleavage sites used for the removal of epitope-binding regions as well as well-characterized proteases, reagents, conditions and protocols are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. The selection, making and use of an appropriate protease cleavage site are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0131] Thus, in an embodiment, an exogenous protease cleavage site is located between an epitope-binding peptide and a modified Clostridial toxin. In other aspects of this embodiment, a bovine enterokinase cleavage site is located between an epitope-binding region and a modified Clostridial toxin, a Tobacco Etch Virus protease cleavage site is located between an epitope-binding region and a modified Clostridial toxin, a Human Rhinovirus 3C protease cleavage site is located between an epitope-binding region and a modified Clostridial toxin, a SUMO/ULP-1 protease cleavage site is located between an epitope-binding region and a modified Clostridial toxin, a Thrombin protease cleavage site is located between an epitope-binding region and a modified Clostridial toxin, or a Coagulation Factor Xa protease cleavage site is located between an epitope-binding region and a modified Clostridial toxin. In other aspects of the embodiment, the bovine enterokinase protease cleavage site located between an epitope-binding region and a modified Clostridial toxin comprises SEQ ID NO: 50. In other aspects of the embodiment, the Tobacco Etch Virus protease cleavage site located between an epitope-binding region and a modified Clostridial toxin comprises SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59 or SEQ ID NO: 60. In still other aspects of the embodiment, the Human Rhinovirus 3C protease cleavage site located between an epitope-binding region and a modified Clostridial toxin comprises SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 66. In yet other aspects of the embodiment, the SUMO/ULP-1 protease cleavage site located between an epitope-binding region and a modified Clostridial toxin comprises SEQ ID NO: 67. In further other aspects of the embodiment, the Thrombin protease cleavage site located between an epitope-binding region and a modified Clostridial

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toxin comprises SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81 or SEQ ID NO: 82. In other aspects of the embodiment, the Coagulation Factor Xa protease cleavage site located between an epitope-binding region and a modified Clostridial toxin comprises SEQ ID NO: 83 or SEQ ID NO: 84.

Table 6: Di-chain Loop Region				
Toxin	SEQ ID NO	Light Chain Region	Di-chain Loop Protease Cleavage Site	Heavy Chain Region
BoNT/A	1	NMNFTKLKNFTGLFEFYKLL	CVRGIITSKTKSLDKGYNK*-----ALNDLC	IKVNNWDL
BoNT/B	2	KQAYEEISKEHLAVYKIQM	CKSVK*-----APGIC	IDVDNEDL
BoNT/C1	3	PALRKVNPNMMLYLFTKF	CHKAIDGRSLYNK*-----TLDC	RELLVKNTDL
BoNT/D	4	PALQKLSSESVDLFTKV	CLRLTKNSR*-----DDSTC	IKVKNNRL
BoNT/E	5	IITPITGRGLVKKIIRF	CKNIVSVKGIR*-----KSIC	IEINNDEL
BoNT/F	6	IIDSIPDKGLVEKIVKF	CKSVIPRKGTK*-----APRLC	IRVNNSEL
BoNT/G	7	KEAYEEISLEHLVIYRIAM	CKPVMYKNTGK*-----SEQC	IIVNNEDL
TeNT	8	TNAFRNVDGSGLVSKLIGL	CKKIIPPTNIRENLYNRTA*SLTDLGGELC	IKIKNEDL

The amino acid sequence displayed are as follows: BoNT/A, residues 325-462 of SEQ ID No: 1; BoNT/B, residues 332-454 of SEQ ID No: 2; BoNT/C1, residues 334-463 of SEQ ID No: 3; BoNT/D, residues 334-458 of SEQ ID No: 4; BoNT/E, residues 311-434 of SEQ ID No: 5; BoNT/F, residues 328-453 of SEQ ID No: 6; BoNT/G, residues 331-458 of SEQ ID No: 7; and TeNT, residues 334-474 of SEQ ID No: 8. An asterisks (*) indicates the peptide bond that is cleaved by a Clostridial toxin protease.

[0132] It is envisioned that an exogenous protease cleavage site can be used to convert the single-chain polypeptide form of a modified Clostridial toxin disclosed in the present specification into the di-chain form. As mentioned above, Clostridial toxins are translated as a single-chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulfide loop by a naturally-occurring protease. This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by a single disulphide bond and noncovalent interactions. While the naturally-occurring protease is currently not known, cleavage occurs within the di-chain loop region between the two cysteine residues that form the disulfide bridge (Table 6). Replacement of the naturally-occurring protease cleavage site with an exogenous protease cleavage site will enable cleavage of a modified Clostridial toxin disclosed in the present specification when expressed in an organism that does not produce the endogenous Clostridial protease used to cleave the di-chain loop region of a toxin.

[0133] Thus in an embodiment, an exogenous protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In aspects of this embodiment, a bovine enterokinase cleavage site is located within the di-chain loop of a modified Clostridial toxin, a Tobacco Etch Virus protease cleavage site is located within the di-chain loop of a modified Clostridial toxin, a Human Rhinovirus 3C protease cleavage site is located within the di-chain loop of a modified Clostridial toxin, a SUMO/ULP-1 protease

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cleavage site is located within the di-chain loop of a modified Clostridial toxin, a Thrombin protease cleavage site is located within the di-chain loop of a modified Clostridial toxin, or a Coagulation Factor Xa protease cleavage site is located within the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, the bovine enterokinase protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 50. In other aspects of the embodiment, the Tobacco Etch Virus protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59 or SEQ ID NO: 60. In still other aspects of the embodiment, the Human Rhinovirus 3C protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 66. In yet other aspects of the embodiment, the SUMO/ULP-1 protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 67. In further other aspects of the embodiment, the Thrombin protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81 or SEQ ID NO: 82. In other aspects of the embodiment, the Coagulation Factor Xa protease cleavage site located within the di-chain loop of a modified Clostridial toxin comprises SEQ ID NO: 83 or SEQ ID NO: 84.

[0134] Aspects of the present invention provide, in part modified Clostridial toxins. Non-limiting examples of Clostridial toxin modifications disclosed in the present specification include, *e.g.*, addition of a PAR ligand domain, addition of a protease cleavage site, rearrangement of the enzymatic, translocation and binding domains, addition of a spacer region and addition of an epitope-binding region. It is understood that all such modifications do not substantially affect the ability of a Clostridial toxin to intoxicate a cell. As used herein, the term "do not substantially affect" means a Clostridial toxin can still execute the overall cellular mechanism whereby a Clostridial toxin enters a neuron and inhibits neurotransmitter release and encompasses the binding of a Clostridial toxin to a low or high affinity receptor complex, the internalization of the toxin/receptor complex, the translocation of the Clostridial toxin light chain into the cytoplasm and the enzymatic modification of a Clostridial toxin substrate. In aspects of this embodiment, the modified Clostridial toxin is, *e.g.*, at least 10% as toxic as a naturally-occurring Clostridial toxin, at least 20% as toxic as a naturally-occurring Clostridial toxin, at least 30% as toxic as a naturally-occurring Clostridial toxin, at least 40% as toxic as a naturally-occurring Clostridial toxin, at least 50% as toxic as a naturally-occurring Clostridial toxin, at least 60% as toxic as a naturally-occurring Clostridial toxin, at least 70% as toxic as a naturally-occurring Clostridial toxin, at least 80% as toxic as a naturally-occurring Clostridial toxin, at least 90% as toxic as a naturally-occurring Clostridial toxin or at least 95% as toxic as a naturally-occurring Clostridial toxin. In aspects of this embodiment, the modified Clostridial toxin is, *e.g.*, at most 10% as toxic as a naturally-occurring Clostridial toxin, at most 20% as toxic as a naturally-occurring Clostridial toxin, at most 30% as toxic as a naturally-occurring Clostridial toxin, at most 40% as toxic as a naturally-occurring Clostridial toxin, at most 50% as toxic as a

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naturally-occurring Clostridial toxin, at most 60% as toxic as a naturally-occurring Clostridial toxin, at most 70% as toxic as a naturally-occurring Clostridial toxin, at most 80% as toxic as a naturally-occurring Clostridial toxin, at most 90% as toxic as a naturally-occurring Clostridial toxin or at most 95% as toxic as a naturally-occurring Clostridial toxin.

[0135] Aspects of the present invention provide, in part polynucleotide molecules. As used herein, the term "polynucleotide molecule" is synonymous with "nucleic acid molecule" and means a polymeric form of nucleotides, such as, *e.g.*, ribonucleotides and deoxyribonucleotides, of any length. It is envisioned that any and all polynucleotide molecules that can encode a modified Clostridial toxin disclosed in the present specification can be useful, including, without limitation naturally-occurring and non-naturally-occurring DNA molecules and naturally-occurring and non-naturally-occurring RNA molecules. Non-limiting examples of naturally-occurring and non-naturally-occurring DNA molecules include single-stranded DNA molecules, double-stranded DNA molecules, genomic DNA molecules, cDNA molecules, vector constructs, such as, *e.g.*, plasmid constructs, phagmid constructs, bacteriophage constructs, retroviral constructs and artificial chromosome constructs. Non-limiting examples of naturally-occurring and non-naturally-occurring RNA molecules include single-stranded RNA, double stranded RNA and mRNA.

[0136] Thus, in an embodiment, a polynucleotide molecule encodes a Clostridial toxin comprises a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain and a Clostridial toxin binding domain. In an aspect of this embodiment, a polynucleotide molecule encodes a Clostridial toxin comprises a naturally occurring Clostridial toxin variant, such as, *e.g.*, a Clostridial toxin isoform or a Clostridial toxin subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a Clostridial toxin comprises a non-naturally occurring Clostridial toxin variant, such as, *e.g.*, a conservative Clostridial toxin variant, a non-conservative Clostridial toxin variant or an active Clostridial toxin fragment, or any combination thereof. In another aspect of this embodiment, a polynucleotide molecule encodes a Clostridial toxin comprises a Clostridial toxin enzymatic domain or an active fragment thereof, a Clostridial toxin translocation domain or an active fragment thereof, a Clostridial toxin binding domain or an active fragment thereof, or any combination thereof. In other aspects of this embodiment, a Clostridial toxins comprises a BoNT/A, a BoNT/B, a BoNT/C1, a BoNT/D, a BoNT/E, a BoNT/F, a BoNT/G or a TeNT.

[0137] In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a BoNT/A. In an aspect of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a BoNT/A enzymatic domain, a BoNT/A translocation domain and a BoNT/A binding domain. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising SEQ ID NO: 1. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a naturally occurring BoNT/A variant, such as, *e.g.*, a BoNT/A isoform or a BoNT/A subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a naturally occurring BoNT/A variant of SEQ ID NO: 1, such as, *e.g.*, a BoNT/A isoform of SEQ ID NO: 1 or a BoNT/A subtype of SEQ

ID NO: 1. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a non-naturally occurring BoNT/A variant, such as, *e.g.*, a conservative BoNT/A variant, a non-conservative BoNT/A variant or an active BoNT/A fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a non-naturally occurring BoNT/A variant of SEQ ID NO: 1, such as, *e.g.*, a conservative BoNT/A variant of SEQ ID NO: 1, a non-conservative BoNT/A variant of SEQ ID NO: 1 or an active BoNT/A fragment of SEQ ID NO: 1, or any combination thereof. In yet another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a BoNT/A enzymatic domain or an active fragment thereof, a BoNT/A translocation domain or an active fragment thereof, a BoNT/A binding domain or an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/A comprising a BoNT/A enzymatic domain of amino acids 1-448 from SEQ ID NO: 1 or an active fragment thereof, a BoNT/A translocation domain of amino acids 449-860 from SEQ ID NO: 1 or an active fragment thereof, a BoNT/A binding domain of amino acids 861-1296 from SEQ ID NO: 1 or an active fragment thereof, and any combination thereof.

[0138] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 1, at least 75% amino acid identity with the SEQ ID NO: 1, at least 80% amino acid identity with SEQ ID NO: 1, at least 85% amino acid identity with SEQ ID NO: 1, at least 90% amino acid identity with SEQ ID NO: 1 or at least 95% amino acid identity with SEQ ID NO: 1. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 1, at most 75% amino acid identity with the SEQ ID NO: 1, at most 80% amino acid identity with SEQ ID NO: 1, at most 85% amino acid identity with SEQ ID NO: 1, at most 90% amino acid identity with SEQ ID NO: 1 or at most 95% amino acid identity with SEQ ID NO: 1.

[0139] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 1. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 1. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 1. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 1. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 1. In other

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aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 1.

[0140] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 1. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 1. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 1. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 1. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 1. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/A comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 1.

[0141] In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a BoNT/B. In an aspect of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a BoNT/B enzymatic domain, a BoNT/B translocation domain and a BoNT/B binding domain. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising SEQ ID NO: 2. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a naturally occurring BoNT/B variant, such as, *e.g.*, a BoNT/B isoform or a BoNT/B subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a naturally occurring BoNT/B variant of SEQ ID NO: 2, such as, *e.g.*, a BoNT/B isoform of SEQ ID NO: 2 or a BoNT/B subtype of SEQ ID NO: 2. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a non-naturally occurring BoNT/B variant, such as, *e.g.*, a conservative BoNT/B variant, a non-conservative BoNT/B variant or an active BoNT/B fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a non-naturally occurring BoNT/B variant of SEQ ID NO: 2, such as, *e.g.*, a conservative BoNT/B variant of SEQ ID NO: 2, a non-conservative BoNT/B variant of SEQ ID NO: 2 or an active BoNT/B fragment of SEQ ID NO: 2, or any combination thereof. In yet another aspect of this embodiment, a BoNT/B comprising a BoNT/B enzymatic domain or an active fragment thereof, a BoNT/B translocation domain or active fragment thereof, a BoNT/B binding domain or active fragment thereof, and any combination thereof. In

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yet another aspect of this embodiment, a BoNT/B comprising a BoNT/B enzymatic domain of amino acids 1-441 from SEQ ID NO: 2 or active fragment thereof, a BoNT/B translocation domain of amino acids 442-847 from SEQ ID NO: 2 or active fragment thereof, a BoNT/B binding domain of amino acids 848-1291 from SEQ ID NO: 2 or active fragment thereof, and any combination thereof.

[0142] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 2, at least 75% amino acid identity with the SEQ ID NO: 2, at least 80% amino acid identity with SEQ ID NO: 2, at least 85% amino acid identity with SEQ ID NO: 2, at least 90% amino acid identity with SEQ ID NO: 2 or at least 95% amino acid identity with SEQ ID NO: 2. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 2, at most 75% amino acid identity with the SEQ ID NO: 2, at most 80% amino acid identity with SEQ ID NO: 2, at most 85% amino acid identity with SEQ ID NO: 2, at most 90% amino acid identity with SEQ ID NO: 2 or at most 95% amino acid identity with SEQ ID NO: 2.

[0143] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 2. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 2. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 2. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 2. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 2. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 2.

[0144] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 2. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 2. In yet other aspects of this embodiment, a

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polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 2. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 2. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 2. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/B comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 2.

[0145] In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a BoNT/C1. In an aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a BoNT/C1 enzymatic domain, a BoNT/C1 translocation domain and a BoNT/C1 binding domain. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising SEQ ID NO: 3. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a naturally occurring BoNT/C1 variant, such as, *e.g.*, a BoNT/C1 isoform or a BoNT/C1 subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a naturally occurring BoNT/C1 variant of SEQ ID NO: 3, such as, *e.g.*, a BoNT/C1 isoform of SEQ ID NO: 3 or a BoNT/C1 subtype of SEQ ID NO: 3. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a non-naturally occurring BoNT/C1 variant, such as, *e.g.*, a conservative BoNT/C1 variant, a non-conservative BoNT/C1 variant or an active BoNT/C1 fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a non-naturally occurring BoNT/C1 variant of SEQ ID NO: 3, such as, *e.g.*, a conservative BoNT/C1 variant of SEQ ID NO: 3, a non-conservative BoNT/C1 variant of SEQ ID NO: 3 or an active BoNT/C1 fragment of SEQ ID NO: 3, or any combination thereof. In yet another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a BoNT/C1 enzymatic domain or active fragment thereof, a BoNT/C1 translocation domain or active fragment thereof, a BoNT/C1 binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a BoNT/C1 enzymatic domain of amino acid 1-449 from SEQ ID NO: 3 or active fragment thereof, a BoNT/C1 translocation domain of amino acids 450-855 from SEQ ID NO: 3 or active fragment thereof, a BoNT/C1 binding domain of amino acids 856-1291 from SEQ ID NO: 3 or active fragment thereof, and any combination thereof.

[0146] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 3, at least 75% amino acid identity with the SEQ ID NO: 3, at least 80% amino acid identity with SEQ ID NO: 3, at least 85% amino acid identity with SEQ ID NO: 3, at least 90% amino acid identity with SEQ ID NO: 3 or at least 95%

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amino acid identity with SEQ ID NO: 3. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 3, at most 75% amino acid identity with the SEQ ID NO: 3, at most 80% amino acid identity with SEQ ID NO: 3, at most 85% amino acid identity with SEQ ID NO: 3, at most 90% amino acid identity with SEQ ID NO: 3 or at most 95% amino acid identity with SEQ ID NO: 3.

[0147] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 3. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 3. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 3. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 3. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 3. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 3.

[0148] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 3. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 3. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 3. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 3. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 3. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/C1 comprising a polypeptide having, *e.g.*, at

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least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 3.

[0149] In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a BoNT/D. In an aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a BoNT/D enzymatic domain, a BoNT/D translocation domain and a BoNT/D binding domain. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising SEQ ID NO: 4. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a naturally occurring BoNT/D variant, such as, *e.g.*, a BoNT/D isoform or a BoNT/D subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a naturally occurring BoNT/D variant of SEQ ID NO: 4, such as, *e.g.*, a BoNT/D isoform of SEQ ID NO: 4 or a BoNT/D subtype of SEQ ID NO: 4. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a non-naturally occurring BoNT/D variant, such as, *e.g.*, a conservative BoNT/D variant, a non-conservative BoNT/D variant or an active BoNT/D fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a non-naturally occurring BoNT/D variant of SEQ ID NO: 4, such as, *e.g.*, a conservative BoNT/D variant of SEQ ID NO: 4, a non-conservative BoNT/D variant of SEQ ID NO: 4 or an active BoNT/D fragment of SEQ ID NO: 4, or any combination thereof. In yet another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a BoNT/D enzymatic domain or an active fragment thereof, a BoNT/D translocation domain or an active fragment thereof, a BoNT/D binding domain or an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/D comprising a BoNT/D enzymatic domain of amino acids 1-442 from SEQ ID NO: 4 or an active fragment thereof, a BoNT/D translocation domain of amino acids 443-851 from SEQ ID NO: 4 or an active fragment thereof, a BoNT/D binding domain of amino acids 852-1276 from SEQ ID NO: 4 or an active fragment thereof, and any combination thereof.

[0150] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 4, at least 75% amino acid identity with the SEQ ID NO: 4, at least 80% amino acid identity with SEQ ID NO: 4, at least 85% amino acid identity with SEQ ID NO: 4, at least 90% amino acid identity with SEQ ID NO: 4 or at least 95% amino acid identity with SEQ ID NO: 4. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 4, at most 75% amino acid identity with the SEQ ID NO: 4, at most 80% amino acid identity with SEQ ID NO: 4, at most 85% amino acid identity with SEQ ID NO: 4, at most 90% amino acid identity with SEQ ID NO: 4 or at most 95% amino acid identity with SEQ ID NO: 4.

[0151] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 4. In other aspects of

this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 4. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 4. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 4. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 4. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 4.

[0152] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 4. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 4. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 4. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 4. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 4. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 4.

[0153] In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a BoNT/E. In an aspect of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a BoNT/E enzymatic domain, a BoNT/E translocation domain and a BoNT/E binding domain. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising SEQ ID NO: 5. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a naturally occurring BoNT/E variant, such as, *e.g.*, a BoNT/E isoform or a BoNT/E subtype. In another aspect of

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this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a naturally occurring BoNT/E variant of SEQ ID NO: 5, such as, *e.g.*, a BoNT/E isoform of SEQ ID NO: 5 or a BoNT/E subtype of SEQ ID NO: 5. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a non-naturally occurring BoNT/E variant, such as, *e.g.*, a conservative BoNT/E variant, a non-conservative BoNT/E variant or an active BoNT/E fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a non-naturally occurring BoNT/E variant of SEQ ID NO: 5, such as, *e.g.*, a conservative BoNT/E variant of SEQ ID NO: 5, a non-conservative BoNT/E variant of SEQ ID NO: 5 or an active BoNT/E fragment of SEQ ID NO: 5, or any combination thereof. In yet another aspect of this embodiment, a BoNT/E comprising a BoNT/E enzymatic domain or an active fragment thereof, a BoNT/E translocation domain or active fragment thereof, a BoNT/E binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a BoNT/E comprising a BoNT/E enzymatic domain of amino acids 1-422 from SEQ ID NO: 5 or active fragment thereof, a BoNT/E translocation domain of amino acids 423-834 from SEQ ID NO: 5 or active fragment thereof, a BoNT/E binding domain of amino acids 835-1252 from SEQ ID NO: 5 or active fragment thereof, and any combination thereof.

[0154] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 5, at least 75% amino acid identity with the SEQ ID NO: 5, at least 80% amino acid identity with SEQ ID NO: 5, at least 85% amino acid identity with SEQ ID NO: 5, at least 90% amino acid identity with SEQ ID NO: 5 or at least 95% amino acid identity with SEQ ID NO: 5. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 5, at most 75% amino acid identity with the SEQ ID NO: 5, at most 80% amino acid identity with SEQ ID NO: 5, at most 85% amino acid identity with SEQ ID NO: 5, at most 90% amino acid identity with SEQ ID NO: 5 or at most 95% amino acid identity with SEQ ID NO: 5.

[0155] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 5. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 5. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 5. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 5. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20,

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30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 5. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 5.

[0156] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 5. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 5. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 5. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 5. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 5. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/E comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 5.

[0157] In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a BoNT/F. In an aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a BoNT/F enzymatic domain, a BoNT/F translocation domain and a BoNT/F binding domain. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising SEQ ID NO: 6. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a naturally occurring BoNT/F variant, such as, *e.g.*, a BoNT/F isoform or a BoNT/F subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a naturally occurring BoNT/F variant of SEQ ID NO: 6, such as, *e.g.*, a BoNT/F isoform of SEQ ID NO: 6 or a BoNT/F subtype of SEQ ID NO: 6. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a non-naturally occurring BoNT/F variant, such as, *e.g.*, a conservative BoNT/F variant, a non-conservative BoNT/F variant or an active BoNT/F fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a non-naturally occurring BoNT/F variant of SEQ ID NO: 6, such as, *e.g.*, a conservative BoNT/F variant of SEQ ID NO: 6, a non-conservative BoNT/F variant of SEQ ID NO: 6 or an active BoNT/F fragment of SEQ ID NO: 6, or any combination thereof. In yet another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a BoNT/F enzymatic domain or active fragment thereof, a BoNT/F translocation

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domain or active fragment thereof, a BoNT/F binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a BoNT/F enzymatic domain of amino acid 1-436 from SEQ ID NO: 6 or active fragment thereof, a BoNT/F translocation domain of amino acids 437-852 from SEQ ID NO: 6 or active fragment thereof, a BoNT/F binding domain of amino acids 853-1274 from SEQ ID NO: 6 or active fragment thereof, and any combination thereof.

[0158] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 6, at least 75% amino acid identity with the SEQ ID NO: 6, at least 80% amino acid identity with SEQ ID NO: 6, at least 85% amino acid identity with SEQ ID NO: 6, at least 90% amino acid identity with SEQ ID NO: 6 or at least 95% amino acid identity with SEQ ID NO: 6. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 6, at most 75% amino acid identity with the SEQ ID NO: 6, at most 80% amino acid identity with SEQ ID NO: 6, at most 85% amino acid identity with SEQ ID NO: 6, at most 90% amino acid identity with SEQ ID NO: 6 or at most 95% amino acid identity with SEQ ID NO: 6.

[0159] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 6. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 6. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 6. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 6. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 6. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 6.

[0160] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 6. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at least

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one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 6. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 6. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 6. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 6. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/F comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 6.

[0161] In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a BoNT/G. In an aspect of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a BoNT/G enzymatic domain, a BoNT/G translocation domain and a BoNT/G binding domain. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising SEQ ID NO: 7. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a naturally occurring BoNT/G variant, such as, *e.g.*, a BoNT/G isoform or a BoNT/G subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a naturally occurring BoNT/G variant of SEQ ID NO: 7, such as, *e.g.*, a BoNT/G isoform of SEQ ID NO: 7 or a BoNT/G subtype of SEQ ID NO: 7. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a non-naturally occurring BoNT/G variant, such as, *e.g.*, a conservative BoNT/G variant, a non-conservative BoNT/G variant or an active BoNT/G fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/D comprising a non-naturally occurring BoNT/G variant of SEQ ID NO: 7, such as, *e.g.*, a conservative BoNT/G variant of SEQ ID NO: 7, a non-conservative BoNT/G variant of SEQ ID NO: 7 or an active BoNT/G fragment of SEQ ID NO: 7, or any combination thereof. In yet another aspect of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a BoNT/G enzymatic domain or an active fragment thereof, a BoNT/G translocation domain or an active fragment thereof, a BoNT/G binding domain or an active fragment thereof, or any combination thereof. In yet another aspect of this embodiment, a BoNT/G comprising a BoNT/G enzymatic domain of amino acids 1-442 from SEQ ID NO: 7 or an active fragment thereof, a BoNT/G translocation domain of amino acids 443-852 from SEQ ID NO: 7 or an active fragment thereof, a BoNT/G binding domain of amino acids 853-1297 from SEQ ID NO: 7 or an active fragment thereof, and any combination thereof.

[0162] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 7, at least 75% amino acid

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identity with the SEQ ID NO: 7, at least 80% amino acid identity with SEQ ID NO: 7, at least 85% amino acid identity with SEQ ID NO: 7, at least 90% amino acid identity with SEQ ID NO: 7 or at least 95% amino acid identity with SEQ ID NO: 7. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 7, at most 75% amino acid identity with the SEQ ID NO: 7, at most 80% amino acid identity with SEQ ID NO: 7, at most 85% amino acid identity with SEQ ID NO: 7, at most 90% amino acid identity with SEQ ID NO: 7 or at most 95% amino acid identity with SEQ ID NO: 7.

[0163] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 7. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 7. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 7. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 7. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 7. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 7.

[0164] In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 7. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 7. In yet other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 7. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 7. In still other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50,

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100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 7. In other aspects of this embodiment, a polynucleotide molecule encodes a BoNT/G comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 7.

[0165] In another embodiment, a polynucleotide molecule encodes a Clostridial toxin comprising a TeNT. In an aspect of this embodiment, a polynucleotide molecule encodes a TeNT comprising a TeNT enzymatic domain, a TeNT translocation domain and a TeNT binding domain. In an aspect of this embodiment, a polynucleotide molecule encodes a TeNT comprising SEQ ID NO: 8. In another aspect of this embodiment, a polynucleotide molecule encodes a TeNT comprising a naturally occurring TeNT variant, such as, *e.g.*, a TeNT isoform or a TeNT subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a TeNT comprising a naturally occurring TeNT variant of SEQ ID NO: 8, such as, *e.g.*, a TeNT isoform of SEQ ID NO: 8 or a TeNT subtype of SEQ ID NO: 8. In still another aspect of this embodiment, a polynucleotide molecule encodes a TeNT comprising a non-naturally occurring TeNT variant, such as, *e.g.*, a conservative TeNT variant, a non-conservative TeNT variant or an active TeNT fragment, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a TeNT comprising a non-naturally occurring TeNT variant of SEQ ID NO: 8, such as, *e.g.*, a conservative TeNT variant of SEQ ID NO: 8, a non-conservative TeNT variant of SEQ ID NO: 8 or an active TeNT fragment of SEQ ID NO: 8, or any combination thereof. In yet another aspect of this embodiment, a TeNT comprising a TeNT enzymatic domain or an active fragment thereof, a TeNT translocation domain or active fragment thereof, a TeNT binding domain or active fragment thereof, and any combination thereof. In yet another aspect of this embodiment, a TeNT comprising a TeNT enzymatic domain of amino acids 1-441 from SEQ ID NO: 8 or active fragment thereof, a TeNT translocation domain of amino acids 442-870 from SEQ ID NO: 8 or active fragment thereof, a TeNT binding domain of amino acids 871-1315 from SEQ ID NO: 8 or active fragment thereof, and any combination thereof.

[0166] In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at least 70% amino acid identity with SEQ ID NO: 8, at least 75% amino acid identity with the SEQ ID NO: 8, at least 80% amino acid identity with SEQ ID NO: 8, at least 85% amino acid identity with SEQ ID NO: 8, at least 90% amino acid identity with SEQ ID NO: 8 or at least 95% amino acid identity with SEQ ID NO: 8. In yet other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at most 70% amino acid identity with SEQ ID NO: 8, at most 75% amino acid identity with the SEQ ID NO: 8, at most 80% amino acid identity with SEQ ID NO: 8, at most 85% amino acid identity with SEQ ID NO: 8, at most 90% amino acid identity with SEQ ID NO: 8 or at most 95% amino acid identity with SEQ ID NO: 8.

[0167] In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50,

100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 8. In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid substitutions relative to SEQ ID NO: 8. In yet other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 8. In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid deletions relative to SEQ ID NO: 8. In still other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 8. In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 non-contiguous amino acid additions relative to SEQ ID NO: 8.

[0168] In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 8. In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid substitutions relative to SEQ ID NO: 8. In yet other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 8. In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid deletions relative to SEQ ID NO: 8. In still other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 8. In other aspects of this embodiment, a polynucleotide molecule encodes a TeNT comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine, 10, 20, 30, 40, 50, 100, 200 or 500 contiguous amino acid additions relative to SEQ ID NO: 8.

[0169] In still another embodiment, a polynucleotide molecule encodes a PAR ligand domain comprising a naturally occurring PAR ligand domain variant, such as, *e.g.*, a PAR ligand domain isoform or a PAR ligand domain subtype. In another embodiment, a polynucleotide molecule encodes a PAR ligand domain comprising a non-naturally occurring PAR ligand domain variant, such as, *e.g.*, a conservative PAR ligand domain variant, a non-conservative PAR ligand domain variant or a PAR ligand domain peptidomimetic, or any combination thereof.

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[0170] In still another embodiment, a polynucleotide molecule encodes a PAR ligand domain comprising a PAR1 ligand domain. In an aspect of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising SEQ ID NO: 13. In another aspect of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a naturally occurring PAR1 ligand domain variant, such as, *e.g.*, a PAR1 ligand domain isoform or a PAR1 ligand domain subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a naturally occurring PAR1 ligand domain variant of SEQ ID NO: 13, such as, *e.g.*, a PAR1 ligand domain isoform of SEQ ID NO: 13 or a PAR1 ligand domain subtype of SEQ ID NO: 13. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a non-naturally occurring PAR1 ligand domain variant, such as, *e.g.*, a conservative PAR1 ligand domain variant, a non-conservative PAR1 ligand domain variant or a PAR1 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a non-naturally occurring PAR1 ligand domain variant of SEQ ID NO: 13, such as, *e.g.*, a conservative PAR1 ligand domain variant of SEQ ID NO: 13, a non-conservative PAR1 ligand domain variant of SEQ ID NO: 13 or a PAR1 ligand domain peptidomimetic of SEQ ID NO: 13, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22 or SEQ ID NO: 23.

[0171] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 13, at least 67% amino acid identity with the SEQ ID NO: 13, or at least 83% amino acid identity with SEQ ID NO: 13. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 13, at most 67% amino acid identity with the SEQ ID NO: 13, at most 83% amino acid identity with SEQ ID NO: 13.

[0172] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 13. In

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still other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 13.

[0173] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at most two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at least two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 13. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 13. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR1 ligand domain comprising a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 13.

[0174] In still another embodiment, a polynucleotide molecule encodes a PAR ligand domain comprising a PAR2 ligand domain. In an aspect of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising SEQ ID NO: 24. In another aspect of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a naturally occurring PAR2 ligand domain variant, such as, *e.g.*, a PAR2 ligand domain isoform or a PAR2 ligand domain subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a naturally occurring PAR2 ligand domain variant of SEQ ID NO: 24, such as, *e.g.*, a PAR2 ligand domain isoform of SEQ ID NO: 24 or a PAR2 ligand domain subtype of SEQ ID NO: 24. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a non-naturally occurring PAR2 ligand domain variant, such as, *e.g.*, a conservative PAR2 ligand domain variant, a non-conservative PAR2 ligand domain variant or a PAR2 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a non-naturally occurring PAR2 ligand domain variant of SEQ ID NO: 24, such as, *e.g.*, a conservative PAR2 ligand domain variant of SEQ ID NO: 24, a non-conservative PAR2 ligand domain variant of SEQ ID NO: 24 or a PAR2 ligand domain peptidomimetic of SEQ ID NO: 24, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising SEQ ID NO: 24 or SEQ ID NO: 25.

[0175] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 24, at least 67%

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amino acid identity with the SEQ ID NO: 24, or at least 83% amino acid identity with SEQ ID NO: 24. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 24, at most 67% amino acid identity with the SEQ ID NO: 24, at most 83% amino acid identity with SEQ ID NO: 24.

[0176] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 24.

[0177] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at most two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at least two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 24. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 24. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR2 ligand domain comprising a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 24.

[0178] In still another embodiment, a polynucleotide molecule encodes a PAR ligand domain comprising a PAR3 ligand domain. In an aspect of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising SEQ ID NO: 26. In another aspect of this embodiment, a polynucleotide

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molecule encodes a PAR3 ligand domain comprising a naturally occurring PAR3 ligand domain variant, such as, *e.g.*, a PAR3 ligand domain isoform or a PAR3 ligand domain subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a naturally occurring PAR3 ligand domain variant of SEQ ID NO: 26, such as, *e.g.*, a PAR3 ligand domain isoform of SEQ ID NO: 26 or a PAR3 ligand domain subtype of SEQ ID NO: 26. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a non-naturally occurring PAR3 ligand domain variant, such as, *e.g.*, a conservative PAR3 ligand domain variant, a non-conservative PAR3 ligand domain variant or a PAR3 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a non-naturally occurring PAR3 ligand domain variant of SEQ ID NO: 26, such as, *e.g.*, a conservative PAR3 ligand domain variant of SEQ ID NO: 26, a non-conservative PAR3 ligand domain variant of SEQ ID NO: 26 or a PAR3 ligand domain peptidomimetic of SEQ ID NO: 26, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising SEQ ID NO: 26 or SEQ ID NO: 27.

[0179] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 26, at least 67% amino acid identity with the SEQ ID NO: 26, or at least 83% amino acid identity with SEQ ID NO: 26. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 26, at most 67% amino acid identity with the SEQ ID NO: 26, at most 83% amino acid identity with SEQ ID NO: 26.

[0180] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 26.

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[0181] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at most two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at least two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 26. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 26. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR3 ligand domain comprising a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 26.

[0182] In still another embodiment, a polynucleotide molecule encodes a PAR ligand domain comprising a PAR4 ligand domain. In an aspect of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising SEQ ID NO: 28. In another aspect of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a naturally occurring PAR4 ligand domain variant, such as, *e.g.*, a PAR4 ligand domain isoform or a PAR4 ligand domain subtype. In another aspect of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a naturally occurring PAR4 ligand domain variant of SEQ ID NO: 28, such as, *e.g.*, a PAR4 ligand domain isoform of SEQ ID NO: 28 or a PAR4 ligand domain subtype of SEQ ID NO: 28. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a non-naturally occurring PAR4 ligand domain variant, such as, *e.g.*, a conservative PAR4 ligand domain variant, a non-conservative PAR4 ligand domain variant or a PAR4 ligand domain peptidomimetic, or any combination thereof. In still another aspect of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a non-naturally occurring PAR4 ligand domain variant of SEQ ID NO: 28, such as, *e.g.*, a conservative PAR4 ligand domain variant of SEQ ID NO: 28, a non-conservative PAR4 ligand domain variant of SEQ ID NO: 28 or a PAR4 ligand domain peptidomimetic of SEQ ID NO: 28, or any combination thereof. In other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46 or SEQ ID NO: 47.

[0183] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at least 50% amino acid identity with SEQ ID NO: 28, at least 67% amino acid identity with the SEQ ID NO: 28, or at least 83% amino acid identity with SEQ ID NO: 28. In

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still other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at most 50% amino acid identity with SEQ ID NO: 28, at most 67% amino acid identity with the SEQ ID NO: 28, at most 83% amino acid identity with SEQ ID NO: 28.

[0184] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three or four non-contiguous amino acid substitutions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at most one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at least one, two, three, four, five, six, seven, eight, nine or ten non-contiguous amino acid additions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at most one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at least one, two or three non-contiguous amino acid deletions relative to SEQ ID NO: 28.

[0185] In other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at most two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at least two, three or four contiguous amino acid substitutions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at most two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 28. In yet other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at least two, three, four, five, six, seven, eight, nine or ten contiguous amino acid additions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at most two or three contiguous amino acid deletions relative to SEQ ID NO: 28. In still other aspects of this embodiment, a polynucleotide molecule encodes a PAR4 ligand domain comprising a polypeptide having, *e.g.*, at least two or three contiguous amino acid deletions relative to SEQ ID NO: 28.

[0186] In yet another embodiment, a polynucleotide molecule encoding a modified Clostridial toxin disclosed in the present specification can further comprise a polynucleotide molecule encoding a flexible region comprising a flexible spacer. In another embodiment, a polynucleotide molecule encoding a modified Clostridial toxin disclosed in the present specification can further comprise a polynucleotide

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molecule encoding a flexible region comprising a plurality of flexible spacers in tandem. In aspects of this embodiment, a polynucleotide molecule encoding a flexible region can comprise in tandem, *e.g.*, at least 1 G-spacer, at least 2 G-spacers, at least 3 G-spacers, at least 4 G-spacers or at least 5 G-spacers. In other aspects of this embodiment, a polynucleotide molecule encoding a flexible region can comprise in tandem, *e.g.*, at most 1 G-spacer, at most 2 G-spacers, at most 3 G-spacers, at most 4 G-spacers or at most 5 G-spacers. In still other aspects of this embodiment, a polynucleotide molecule encoding a flexible region can comprise in tandem, *e.g.*, at least 1 A-spacer, at least 2 A-spacers, at least 3 A-spacers, at least 4 A-spacers or at least 5 A-spacers. In still other aspects of this embodiment, a polynucleotide molecule encoding a flexible region can comprise in tandem, *e.g.*, at most 1 A-spacer, at most 2 A-spacers, at most 3 A-spacers, at most 4 A-spacers or at most 5 A-spacers. In another aspect of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin can comprise a polynucleotide molecule encoding a flexible region comprising one or more copies of the same flexible spacers, one or more copies of different flexible-spacers region, or any combination thereof.

[0187] In yet another embodiment, a polynucleotide molecule encoding a modified Clostridial toxin disclosed in the present specification can further comprises a polynucleotide molecule encoding an epitope-binding region. In another embodiment, a polynucleotide molecule encoding a modified Clostridial toxin disclosed in the present specification can further comprises a polynucleotide molecule encoding a plurality of epitope-binding regions. In aspects of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin can comprise, *e.g.*, at least 1 polynucleotide molecule encoding an epitope-binding region, at least 2 polynucleotide molecules encoding epitope-binding regions, at least 3 polynucleotide molecules encoding epitope-binding regions, at least 4 polynucleotide molecules encoding epitope-binding regions or at least 5 polynucleotide molecules encoding epitope-binding regions. In other aspects of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin can comprise, *e.g.*, at most 1 polynucleotide molecule encoding an epitope-binding region, at most 2 polynucleotide molecules encoding epitope-binding regions, at most 3 polynucleotide molecules encoding epitope-binding regions, at most 4 polynucleotide molecules encoding epitope-binding regions or at most 5 polynucleotide molecules encoding epitope-binding regions. In another aspect of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin can comprise one or more copies of the same polynucleotide molecules encoding epitope-binding region, one or more copies of different polynucleotide molecules encoding epitope-binding region, or any combination thereof. The location of a polynucleotide molecule encoding an epitope-binding region can be in various positions, including, without limitation, at the amino terminus of a modified Clostridial toxin, within a modified Clostridial toxin, or at the carboxyl terminus of a modified Clostridial toxin.

[0188] In yet another embodiment, polynucleotide molecules encoding a modified Clostridial toxin disclosed in the present specification can further comprise a polynucleotide molecule encoding an exogenous protease cleavage site. In another embodiment, a polynucleotide molecule encoding a modified Clostridial toxin disclosed in the present specification can further comprises a plurality of

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polynucleotide molecules encoding exogenous protease cleavage sites. In aspects of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin can comprise, *e.g.*, at least 1 polynucleotide molecule encoding an exogenous protease cleavage site, at least 2 polynucleotide molecules encoding exogenous protease cleavage sites, at least 3 polynucleotide molecules encoding exogenous protease cleavage sites, at least 4 polynucleotide molecules encoding exogenous protease cleavage sites or at least 5 polynucleotide molecules encoding exogenous protease cleavage sites. In other aspects of this embodiment, polynucleotide molecules encoding a modified Clostridial toxin can comprise, *e.g.*, at most 1 polynucleotide molecule encoding an exogenous protease cleavage site, at most 2 polynucleotide molecules encoding exogenous protease cleavage sites, at most 3 polynucleotide molecules encoding exogenous protease cleavage sites, at most 4 polynucleotide molecules encoding exogenous protease cleavage sites or at most 5 polynucleotide molecules encoding exogenous protease cleavage sites. In another aspect of this embodiment, a polynucleotide molecule encoding a modified Clostridial toxin can comprise one or more copies of the same exogenous protease cleavage site, one or more copies of different exogenous protease cleavage site, or any combination thereof.

[0189] In yet another embodiment, a polynucleotide molecule encoding an exogenous protease cleavage site is located between a polynucleotide molecule encoding an epitope-binding peptide and a polynucleotide molecule encoding a modified Clostridial toxin. In other aspects of this embodiment, a polynucleotide molecule encoding a bovine enterokinase cleavage site is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin, a polynucleotide molecule encoding a Tobacco Etch Virus protease cleavage site is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin, a polynucleotide molecule encoding a Human Rhinovirus 3C protease cleavage site is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin, a polynucleotide molecule encoding a SUMO/ULP-1 protease cleavage site is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin, a polynucleotide molecule encoding a Thrombin protease cleavage site is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin, or a polynucleotide molecule encoding a Coagulation Factor Xa protease cleavage site is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin. In other aspects of the embodiment, a polynucleotide molecule encoding the bovine enterokinase protease cleavage site of SEQ ID NO: 50 is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin. In other aspects of the embodiment, a polynucleotide molecule encoding the Tobacco Etch Virus protease cleavage site of SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59 or SEQ ID NO: 60 is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin. In still other

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aspects of the embodiment, a polynucleotide molecule encoding the Human Rhinovirus 3C protease cleavage site of SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 66 is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin. In yet other aspects of the embodiment, a polynucleotide molecule encoding the SUMO/ULP-1 protease cleavage site of SEQ ID NO: 67 is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin. In further other aspects of the embodiment, a polynucleotide molecule encoding the Thrombin protease cleavage site of SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81 or SEQ ID NO: 82 is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin. In other aspects of the embodiment, a polynucleotide molecule encoding the Coagulation Factor Xa protease cleavage site of SEQ ID NO: 83 or SEQ ID NO: 84 is located between a polynucleotide molecule encoding an epitope-binding region and a polynucleotide molecule encoding a modified Clostridial toxin.

[0190] In yet another embodiment, a polynucleotide molecule encoding an exogenous protease cleavage site is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin. In aspects of this embodiment, a polynucleotide molecule encoding a bovine enterokinase cleavage site is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin, a polynucleotide molecule encoding a Tobacco Etch Virus protease cleavage site is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin, a polynucleotide molecule encoding a Human Rhinovirus 3C protease cleavage site is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin, a polynucleotide molecule encoding a SUMO/ULP-1 protease cleavage site is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin, a polynucleotide molecule encoding a Thrombin protease cleavage site is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin, or a polynucleotide molecule encoding a Coagulation Factor Xa protease cleavage site is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, a polynucleotide molecule encoding the bovine enterokinase protease cleavage site of SEQ ID NO: 50 is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, a polynucleotide molecule encoding the Tobacco Etch Virus protease cleavage site of SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59 or SEQ ID NO: 60 is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin. In still other aspects of the embodiment, a polynucleotide molecule encoding the Human Rhinovirus 3C protease cleavage site of SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65 or SEQ ID NO: 66 is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin. In yet other aspects of the

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embodiment, a polynucleotide molecule encoding the SUMO/ULP-1 protease cleavage site of SEQ ID NO: 67 is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin. In further other aspects of the embodiment, a polynucleotide molecule encoding the Thrombin protease cleavage site of SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81 or SEQ ID NO: 82 is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin. In other aspects of the embodiment, a polynucleotide molecule encoding the Coagulation Factor Xa protease cleavage site of SEQ ID NO: 83 or SEQ ID NO: 84 is located within a polynucleotide molecule encoding the di-chain loop of a modified Clostridial toxin.

[0191] Another aspect of the present invention provides a method of producing a modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain, such method comprising the step of expressing a polynucleotide molecule encoding a modified Clostridial toxin in a cell. Another aspect of the present invention provides a method of producing a modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain, such method comprising the steps of introducing an expression construct comprising a polynucleotide molecule encoding a modified Clostridial toxin into a cell and expressing the expression construct in the cell.

[0192] The methods disclosed in the present specification include, in part, a Clostridial toxin. It is envisioned that any and all Clostridial toxins disclosed in the present specification can be produced using the methods disclosed in the present specification. Thus, aspects of this embodiment include producing, without limitation, naturally occurring Clostridial toxins, naturally occurring Clostridial toxins variants, such as, *e.g.*, Clostridial toxins isoforms and Clostridial toxins subtypes, non-naturally occurring Clostridial toxins variants, such as, *e.g.*, conservative Clostridial toxins variants, non-conservative Clostridial toxins variants and Clostridial toxins fragments thereof, or any combination thereof.

[0193] The methods disclosed in the present specification include, in part, a PAR binding domain. It is envisioned that any and all PAR binding domains disclosed in the present specification can be produced using the methods disclosed in the present specification. Thus, aspects of this embodiment include producing, without limitation, naturally occurring PAR binding domains, naturally occurring PAR binding domain variants, such as, *e.g.*, PAR binding domain isoforms and PAR binding domain subtypes, non-naturally occurring PAR binding domain variants, such as, *e.g.*, conservative PAR binding domain variants, non-conservative PAR binding domain variants and PAR binding domain fragments thereof, or any combination thereof.

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[0194] The methods disclosed in the present specification include, in part, a polynucleotide molecule. It is envisioned that any and all polynucleotide molecules disclosed in the present specification can be used. Thus, aspects of this embodiment include, without limitation, polynucleotide molecules encoding naturally occurring Clostridial toxins; polynucleotide molecules encoding naturally occurring Clostridial toxins variants, such as, *e.g.*, Clostridial toxins isoforms and Clostridial toxins subtypes; polynucleotide molecules encoding non-naturally occurring Clostridial toxins variants, such as, *e.g.*, conservative Clostridial toxins variants, non-conservative Clostridial toxins variants and Clostridial toxins fragments thereof, or any combination thereof.

[0195] The methods disclosed in the present specification include, in part, an expression construct. An expression construct comprises a polynucleotide molecule disclosed in the present specification operably-linked to an expression vector useful for expressing the polynucleotide molecule in a cell or cell-free extract. A wide variety of expression vectors can be employed for expressing a polynucleotide molecule encoding a modified Clostridial toxin, including, without limitation, a viral expression vector; a prokaryotic expression vector; eukaryotic expression vectors, such as, *e.g.*, a yeast expression vector, an insect expression vector and a mammalian expression vector; and a cell-free extract expression vector. It is further understood that expression vectors useful to practice aspects of these methods may include those which express a modified Clostridial toxin under control of a constitutive, tissue-specific, cell-specific or inducible promoter element, enhancer element or both. Non-limiting examples of expression vectors, along with well-established reagents and conditions for making and using an expression construct from such expression vectors are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; EMD Biosciences-Novagen, Madison, WI; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. The selection, making and use of an appropriate expression vector are routine procedures well within the scope of one skilled in the art and from the teachings herein.

[0196] Thus, aspects of this embodiment include, without limitation, a viral expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; a prokaryotic expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; a yeast expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; an insect expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin; and a mammalian expression vector operably-linked to a polynucleotide molecule encoding a modified Clostridial toxin. Other aspects of this embodiment include, without limitation, expression constructs suitable for expressing a modified Clostridial toxin disclosed in the present specification using a cell-free extract comprising a cell-free extract expression vector operably linked to a polynucleotide molecule encoding a modified Clostridial toxin. Other aspects of this embodiment include, without limitation, expression constructs comprising polynucleotide molecules comprising any one of SEQ ID NO: 109 through SEQ ID NO: 132 and SEQ ID NO: 136 through SEQ ID NO: 159. Other aspects of this

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embodiment include, without limitation, expression constructs comprising polynucleotide molecules encoding a modified Clostridial toxin comprising any one of SEQ ID NO: 85 through SEQ ID NO: 108.

[0197] The methods disclosed in the present specification include, in part, a cell. It is envisioned that any and all cells can be used. Thus, aspects of this embodiment include, without limitation, prokaryotic cells including, without limitation, strains of aerobic, microaerophilic, capnophilic, facultative, anaerobic, gram-negative and gram-positive bacterial cells such as those derived from, *e.g.*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacteroides fragilis*, *Clostridia perfringens*, *Clostridia difficile*, *Caulobacter crescentus*, *Lactococcus lactis*, *Methylobacterium extorquens*, *Neisseria meningitidis*, *Neisseria meningitidis*, *Pseudomonas fluorescens* and *Salmonella typhimurium*; and eukaryotic cells including, without limitation, yeast strains, such as, *e.g.*, those derived from *Pichia pastoris*, *Pichia methanolica*, *Pichia angusta*, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica*; insect cells and cell lines derived from insects, such as, *e.g.*, those derived from *Spodoptera frugiperda*, *Trichoplusia ni*, *Drosophila melanogaster* and *Manduca sexta*; and mammalian cells and cell lines derived from mammalian cells, such as, *e.g.*, those derived from mouse, rat, hamster, porcine, bovine, equine, primate and human. Cell lines may be obtained from the American Type Culture Collection (2004), at URL address www.atcc.org; European Collection of Cell Cultures (2204), at URL address www.ecacc.org.uk; and the German Collection of Microorganisms and Cell Cultures (2004), at URL address www.dsmz.de. Non-limiting examples of specific protocols for selecting, making and using an appropriate cell line are described in *e.g.*, INSECT CELL CULTURE ENGINEERING (Mattheus F. A. Goosen *et al.* eds., Marcel Dekker, 1993); INSECT CELL CULTURES: FUNDAMENTAL AND APPLIED ASPECTS (J. M. Vlak *et al.* eds., Kluwer Academic Publishers, 1996); Maureen A. Harrison & Ian F. Rae, GENERAL TECHNIQUES OF CELL CULTURE (Cambridge University Press, 1997); CELL AND TISSUE CULTURE: LABORATORY PROCEDURES (Alan Doyle *et al.* eds., John Wiley and Sons, 1998); R. Ian Freshney, CULTURE OF ANIMAL CELLS: A MANUAL OF BASIC TECHNIQUE (Wiley-Liss, 4th ed. 2000); ANIMAL CELL CULTURE: A PRACTICAL APPROACH (John R. W. Masters ed., Oxford University Press, 3rd ed. 2000); MOLECULAR CLONING A LABORATORY MANUAL, *supra*, (2001); BASIC CELL CULTURE: A PRACTICAL APPROACH (John M. Davis, Oxford Press, 2nd ed. 2002); and CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, *supra*, (2004). These protocols are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0198] The methods disclosed in the present specification include, in part, introducing into a cell a polynucleotide molecule. A polynucleotide molecule introduced into a cell can be transiently or stably maintained by that cell. Stably-maintained polynucleotide molecules may be extra-chromosomal and replicate autonomously, or they may be integrated into the chromosomal material of the cell and replicate non-autonomously. It is envisioned that any and all methods for introducing a polynucleotide molecule disclosed in the present specification into a cell can be used. Methods useful for introducing a nucleic acid molecule into a cell include, without limitation, chemical-mediated transfection such as, *e.g.*, calcium phosphate-mediated, diethyl-aminoethyl (DEAE) dextran-mediated, lipid-mediated, polyethyleneimine (PEI)-mediated, polylysine-mediated and polybrene-mediated; physical-mediated transfection, such as,

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e.g., biolistic particle delivery, microinjection, protoplast fusion and electroporation; and viral-mediated transfection, such as, *e.g.*, retroviral-mediated transfection, see, *e.g.*, *Introducing Cloned Genes into Cultured Mammalian Cells*, pp. 16.1-16.62 (Sambrook & Russell, eds., *Molecular Cloning A Laboratory Manual*, Vol. 3, 3rd ed. 2001). One skilled in the art understands that selection of a specific method to introduce an expression construct into a cell will depend, in part, on whether the cell will transiently contain an expression construct or whether the cell will stably contain an expression construct. These protocols are routine procedures within the scope of one skilled in the art and from the teaching herein.

[0199] In an aspect of this embodiment, a chemical-mediated method, termed transfection, is used to introduce a polynucleotide molecule encoding a modified Clostridial toxin into a cell. In chemical-mediated methods of transfection the chemical reagent forms a complex with the nucleic acid that facilitates its uptake into the cells. Such chemical reagents include, without limitation, calcium phosphate-mediated, see, *e.g.*, Martin Jordan & Florian Worm, *Transfection of adherent and suspended cells by calcium phosphate*, 33(2) *Methods* 136-143 (2004); diethyl-aminoethyl (DEAE) dextran-mediated, lipid-mediated, cationic polymer-mediated like polyethyleneimine (PEI)-mediated and polylysine-mediated and polybrene-mediated, see, *e.g.*, Chun Zhang et al., *Polyethylenimine strategies for plasmid delivery to brain-derived cells*, 33(2) *Methods* 144-150 (2004). Such chemical-mediated delivery systems can be prepared by standard methods and are commercially available, see, *e.g.*, CellPfect Transfection Kit (Amersham Biosciences, Piscataway, NJ); Mammalian Transfection Kit, Calcium phosphate and DEAE Dextran, (Stratagene, Inc., La Jolla, CA); Lipofectamine™ Transfection Reagent (Invitrogen, Inc., Carlsbad, CA); ExGen 500 Transfection kit (Fermentas, Inc., Hanover, MD), and SuperFect and Effectene Transfection Kits (Qiagen, Inc., Valencia, CA).

[0200] In another aspect of this embodiment, a physical-mediated method is used to introduce a polynucleotide molecule encoding a modified Clostridial toxin into a cell. Physical techniques include, without limitation, electroporation, biolistic and microinjection. Biolistics and microinjection techniques perforate the cell wall in order to introduce the nucleic acid molecule into the cell, see, *e.g.*, Jeike E. Biewenga et al., *Plasmid-mediated gene transfer in neurons using the biolistics technique*, 71(1) *J. Neurosci. Methods*. 67-75 (1997); and John O'Brien & Sarah C. R. Lummis, *Biolistic and diolistic transfection: using the gene gun to deliver DNA and lipophilic dyes into mammalian cells*, 33(2) *Methods* 121-125 (2004). Electroporation, also termed electroporabilization, uses brief, high-voltage, electrical pulses to create transient pores in the membrane through which the nucleic acid molecules enter and can be used effectively for stable and transient transfections of all cell types, see, *e.g.*, M. Golzio et al., *In vitro and in vivo electric field-mediated permeabilization, gene transfer, and expression*, 33(2) *Methods* 126-135 (2004); and Oliver Greschet al., *New non-viral method for gene transfer into primary cells*, 33(2) *Methods* 151-163 (2004).

[0201] In another aspect of this embodiment, a viral-mediated method, termed transduction, is used to introduce a polynucleotide molecule encoding a modified Clostridial toxin into a cell. In viral-mediated

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methods of transient transduction, the process by which viral particles infect and replicate in a host cell has been manipulated in order to use this mechanism to introduce a nucleic acid molecule into the cell. Viral-mediated methods have been developed from a wide variety of viruses including, without limitation, retroviruses, adenoviruses, adeno-associated viruses, herpes simplex viruses, picornaviruses, alphaviruses and baculoviruses, see, *e.g.*, Armin Blesch, Lentiviral and MLV based retroviral vectors for ex vivo and in vivo gene transfer, 33(2) *Methods* 164-172 (2004); and Maurizio Federico, From lentiviruses to lentivirus vectors, 229 *Methods Mol. Biol.* 3-15 (2003); E. M. Poeschla, Non-primate lentiviral vectors, 5(5) *Curr. Opin. Mol. Ther.* 529-540 (2003); Karim Benihoud *et al.*, Adenovirus vectors for gene delivery, 10(5) *Curr. Opin. Biotechnol.* 440-447 (1999); H. Bueler, Adeno-associated viral vectors for gene transfer and gene therapy, 380(6) *Biol. Chem.* 613-622 (1999); Chooi M. Lai *et al.*, Adenovirus and adeno-associated virus vectors, 21(12) *DNA Cell Biol.* 895-913 (2002); Edward A. Burton *et al.*, Gene delivery using herpes simplex virus vectors, 21(12) *DNA Cell Biol.* 915-936 (2002); Paola Grandi *et al.*, Targeting HSV amplicon vectors, 33(2) *Methods* 179-186 (2004); Ilya Frolov *et al.*, Alphavirus-based expression vectors: strategies and applications, 93(21) *Proc. Natl. Acad. Sci. U. S. A.* 11371-11377 (1996); Markus U. Ehrenguber, Alphaviral gene transfer in neurobiology, 59(1) *Brain Res. Bull.* 13-22 (2002); Thomas A. Kost & J. Patrick Condreay, Recombinant baculoviruses as mammalian cell gene-delivery vectors, 20(4) *Trends Biotechnol.* 173-180 (2002); and A. Huser & C. Hofmann, Baculovirus vectors: novel mammalian cell gene-delivery vehicles and their applications, 3(1) *Am. J. Pharmacogenomics* 53-63 (2003).

[0202] Adenoviruses, which are non-enveloped, double-stranded DNA viruses, are often selected for mammalian cell transduction because adenoviruses handle relatively large polynucleotide molecules of about 36 kb, are produced at high titer, and can efficiently infect a wide variety of both dividing and non-dividing cells, see, *e.g.*, Wim T. J. M. C. Hermens *et al.*, Transient gene transfer to neurons and glia: analysis of adenoviral vector performance in the CNS and PNS, 71(1) *J. Neurosci. Methods* 85-98 (1997); and Hiroyuki Mizuguchi *et al.*, Approaches for generating recombinant adenovirus vectors, 52(3) *Adv. Drug Deliv. Rev.* 165-176 (2001). Transduction using adenoviral-based system do not support prolonged protein expression because the nucleic acid molecule is carried from an episome in the cell nucleus, rather than being integrated into the host cell chromosome. Adenoviral vector systems and specific protocols for how to use such vectors are disclosed in, *e.g.*, ViraPower™ Adenoviral Expression System (Invitrogen, Inc., Carlsbad, CA) and ViraPower™ Adenoviral Expression System Instruction Manual 25-0543 version A, Invitrogen, Inc., (Jul. 15, 2002); and AdEasy™ Adenoviral Vector System (Stratagene, Inc., La Jolla, CA) and AdEasy™ Adenoviral Vector System Instruction Manual 064004f, Stratagene, Inc..

[0203] Nucleic acid molecule delivery can also use single-stranded RNA retroviruses, such as, *e.g.*, oncoretroviruses and lentiviruses. Retroviral-mediated transduction often produce transduction efficiencies close to 100%, can easily control the proviral copy number by varying the multiplicity of infection (MOI), and can be used to either transiently or stably transduce cells, see, *e.g.*, Tiziana Tonini *et al.*, Transient production of retroviral- and lentiviral-based vectors for the transduction of Mammalian cells,

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285 Methods Mol. Biol. 141-148 (2004); Armin Blesch, Lentiviral and MLV based retroviral vectors for ex vivo and in vivo gene transfer, 33(2) Methods 164-172 (2004); Félix Recillas-Targa, Gene transfer and expression in mammalian cell lines and transgenic animals, 267 Methods Mol. Biol. 417-433 (2004); and Roland Wolkowicz *et al.*, Lentiviral vectors for the delivery of DNA into mammalian cells, 246 Methods Mol. Biol. 391-411 (2004). Retroviral particles consist of an RNA genome packaged in a protein capsid, surrounded by a lipid envelope. The retrovirus infects a host cell by injecting its RNA into the cytoplasm along with the reverse transcriptase enzyme. The RNA template is then reverse transcribed into a linear, double stranded cDNA that replicates itself by integrating into the host cell genome. Viral particles are spread both vertically (from parent cell to daughter cells via the provirus) as well as horizontally (from cell to cell via virions). This replication strategy enables long-term persistent expression since the nucleic acid molecules of interest are stably integrated into a chromosome of the host cell, thereby enabling long-term expression of the protein. For instance, animal studies have shown that lentiviral vectors injected into a variety of tissues produced sustained protein expression for more than 1 year, see, *e.g.*, Luigi Naldini *et al.*, In vivo gene delivery and stable transduction of non-dividing cells by a lentiviral vector, 272(5259) Science 263-267 (1996). The Oncoretroviruses-derived vector systems, such as, *e.g.*, Moloney murine leukemia virus (MoMLV), are widely used and infect many different non-dividing cells. Lentiviruses can also infect many different cell types, including dividing and non-dividing cells and possess complex envelope proteins, which allows for highly specific cellular targeting.

[0204] Retroviral vectors and specific protocols for how to use such vectors are disclosed in, *e.g.*, U.S. Patent Nos. Manfred Gossen & Hermann Bujard, Tight control of gene expression in eukaryotic cells by tetracycline-responsive promoters, U.S. Patent No. 5,464,758 (Nov. 7, 1995) and Hermann Bujard & Manfred Gossen, Methods for regulating gene expression, U.S. Patent No. 5,814,618 (Sep. 29, 1998) David S. Hogness, Polynucleotides encoding insect steroid hormone receptor polypeptides and cells transformed with same, U.S. Patent No. 5,514,578 (May 7, 1996) and David S. Hogness, Polynucleotide encoding insect ecdysone receptor, U.S. Patent 6,245,531 (Jun. 12, 2001); Elisabetta Vegeto *et al.*, Progesterone receptor having C. terminal hormone binding domain truncations, U.S. Patent No. 5,364,791 (Nov. 15, 1994), Elisabetta Vegeto *et al.*, Mutated steroid hormone receptors, methods for their use and molecular switch for gene therapy, U.S. Patent No. 5,874,534 (Feb. 23, 1999) and Elisabetta Vegeto *et al.*, Mutated steroid hormone receptors, methods for their use and molecular switch for gene therapy, U.S. Patent No. 5,935,934 (Aug. 10, 1999). Furthermore, such viral delivery systems can be prepared by standard methods and are commercially available, see, *e.g.*, BD™ Tet-Off and Tet-On Gene Expression Systems (BD Biosciences-Clontech, Palo Alto, CA) and BD™ Tet-Off and Tet-On Gene Expression Systems User Manual, PT3001-1, BD Biosciences Clontech, (Mar. 14, 2003), GeneSwitch™ System (Invitrogen, Inc., Carlsbad, CA) and GeneSwitch™ System A Mifepristone-Regulated Expression System for Mammalian Cells version D, 25-0313, Invitrogen, Inc., (Nov. 4, 2002); ViraPower™ Lentiviral Expression System (Invitrogen, Inc., Carlsbad, CA) and ViraPower™ Lentiviral Expression System Instruction Manual 25-0501 version E, Invitrogen, Inc., (Dec. 8, 2003); and Complete Control® Retroviral

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Inducible Mammalian Expression System (Stratagene, La Jolla, CA) and Complete Control[®] Retroviral Inducible Mammalian Expression System Instruction Manual, 064005e.

[0205] The methods disclosed in the present specification include, in part, expressing a modified Clostridial toxin from a polynucleotide molecule. It is envisioned that any of a variety of expression systems may be useful for expressing a modified Clostridial toxin from a polynucleotide molecule disclosed in the present specification, including, without limitation, cell-based systems and cell-free expression systems. Cell-based systems include, without limitation, viral expression systems, prokaryotic expression systems, yeast expression systems, baculoviral expression systems, insect expression systems and mammalian expression systems. Cell-free systems include, without limitation, wheat germ extracts, rabbit reticulocyte extracts and *E. coli* extracts and generally are equivalent to the method disclosed herein. Expression of a polynucleotide molecule using an expression system can include any of a variety of characteristics including, without limitation, inducible expression, non-inducible expression, constitutive expression, viral-mediated expression, stably-integrated expression, and transient expression. Expression systems that include well-characterized vectors, reagents, conditions and cells are well-established and are readily available from commercial vendors that include, without limitation, Ambion, Inc. Austin, TX; BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc. Carlsbad, CA; QIAGEN, Inc., Valencia, CA; Roche Applied Science, Indianapolis, IN; and Stratagene, La Jolla, CA. Non-limiting examples on the selection and use of appropriate heterologous expression systems are described in *e.g.*, PROTEIN EXPRESSION. A PRACTICAL APPROACH (S. J. Higgins and B. David Hames eds., Oxford University Press, 1999); Joseph M. Fernandez & James P. Hoeffler, GENE EXPRESSION SYSTEMS. USING NATURE FOR THE ART OF EXPRESSION (Academic Press, 1999); and Meena Rai & Harish Padh, *Expression Systems for Production of Heterologous Proteins*, 80(9) CURRENT SCIENCE 1121-1128, (2001). These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[0206] A variety of cell-based expression procedures are useful for expressing a modified Clostridial toxin encoded by polynucleotide molecule disclosed in the present specification. Examples included, without limitation, viral expression systems, prokaryotic expression systems, yeast expression systems, baculoviral expression systems, insect expression systems and mammalian expression systems. Viral expression systems include, without limitation, the ViraPower[™] Lentiviral (Invitrogen, Inc., Carlsbad, CA), the Adenoviral Expression Systems (Invitrogen, Inc., Carlsbad, CA), the AdEasy[™] XL Adenoviral Vector System (Stratagene, La Jolla, CA) and the ViraPort[®] Retroviral Gene Expression System (Stratagene, La Jolla, CA). Non-limiting examples of prokaryotic expression systems include the Champion[™] pET Expression System (EMD Biosciences-Novagen, Madison, WI), the TriEx[™] Bacterial Expression Systems (EMD Biosciences-Novagen, Madison, WI), the QIAexpress[®] Expression System (QIAGEN, Inc.), and the Affinity[®] Protein Expression and Purification System (Stratagene, La Jolla, CA). Yeast expression systems include, without limitation, the EasySelect[™] *Pichia* Expression Kit (Invitrogen, Inc., Carlsbad, CA), the YES-Echo[™] Expression Vector Kits (Invitrogen, Inc., Carlsbad, CA) and the

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SpECTRA™ *S. pombe* Expression System (Invitrogen, Inc., Carlsbad, CA). Non-limiting examples of baculoviral expression systems include the BaculoDirect™ (Invitrogen, Inc., Carlsbad, CA), the Bac-to-Bac® (Invitrogen, Inc., Carlsbad, CA), and the BD BaculoGold™ (BD Biosciences-Pharmingen, San Diego, CA). Insect expression systems include, without limitation, the *Drosophila* Expression System (DES®) (Invitrogen, Inc., Carlsbad, CA), InsectSelect™ System (Invitrogen, Inc., Carlsbad, CA) and InsectDirect™ System (EMD Biosciences-Novagen, Madison, WI). Non-limiting examples of mammalian expression systems include the T-REx™ (Tetracycline-Regulated Expression) System (Invitrogen, Inc., Carlsbad, CA), the Flp-In™ T-REx™ System (Invitrogen, Inc., Carlsbad, CA), the pcDNA™ system (Invitrogen, Inc., Carlsbad, CA), the pSecTag2 system (Invitrogen, Inc., Carlsbad, CA), the Exchanger® System, InterPlay™ Mammalian TAP System (Stratagene, La Jolla, CA), Complete Control® Inducible Mammalian Expression System (Stratagene, La Jolla, CA) and LacSwitch® II Inducible Mammalian Expression System (Stratagene, La Jolla, CA).

[0207] Another procedure of expressing a modified Clostridial toxin encoded by polynucleotide molecule disclosed in the present specification employs a cell-free expression system such as, without limitation, prokaryotic extracts and eukaryotic extracts. Non-limiting examples of prokaryotic cell extracts include the RTS 100 *E. coli* HY Kit (Roche Applied Science, Indianapolis, IN), the ActivePro In Vitro Translation Kit (Ambion, Inc., Austin, TX), the EcoPro™ System (EMD Biosciences-Novagen, Madison, WI) and the Expressway™ Plus Expression System (Invitrogen, Inc., Carlsbad, CA). Eukaryotic cell extract include, without limitation, the RTS 100 Wheat Germ CECF Kit (Roche Applied Science, Indianapolis, IN), the TnT® Coupled Wheat Germ Extract Systems (Promega Corp., Madison, WI), the Wheat Germ IVT™ Kit (Ambion, Inc., Austin, TX), the Retic Lysate IVT™ Kit (Ambion, Inc., Austin, TX), the PROTEINscript® II System (Ambion, Inc., Austin, TX) and the TnT® Coupled Reticulocyte Lysate Systems (Promega Corp., Madison, WI).

EXAMPLES

[0208] The following non-limiting examples are provided for illustrative purposes only in order to facilitate a more complete understanding of disclosed embodiments and are in no way intended to limit any of the embodiments disclosed in the present specification.

Example 1

Construction of BoNT/A-ED-PAR1Tb

[0209] This example illustrates how to make a modified Clostridial toxin comprising a PAR binding domain located at the amino terminus of the light chain comprising the enzymatic domain.

[0210] A polynucleotide molecule (SEQ ID NO: 109) based on BoNT/A-ED-PAR1Tb (SEQ ID NO: 85) is synthesized using standard procedures (BlueHeron® Biotechnology, Bothell, WA). Oligonucleotides of 20

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to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-ED-PAR1Tb. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA).

[0211] If desired, an expression optimized polynucleotide molecule (SEQ ID NO: 136) based on BoNT/A-ED-PAR1Tb (SEQ ID NO: 85) can be synthesized in order to improve expression in an *Escherichia coli* strain. The polynucleotide molecule encoding the BoNT/A-ED-PAR1Tb can be modified to 1) contain synonymous codons typically present in native polynucleotide molecules of an *Escherichia coli* strain; 2) contain a G+C content that more closely matches the average G+C content of native polynucleotide molecules found in an *Escherichia coli* strain; 3) reduce polymononucleotide regions found within the polynucleotide molecule; and/or 4) eliminate internal regulatory or structural sites found within the polynucleotide molecule, see, *e.g.*, Lance E. Steward *et al.* Optimizing Expression of Active Botulinum Toxin Type E, PCT Patent Serial No. 2005/020578 (Jun. 9, 2005); Lance E. Steward *et al.* Optimizing Expression of Active Botulinum Toxin Type A, PCT Patent Serial No. 2005/XXXXXX (Aug. 3, 2005). Once sequence optimization is complete, oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-ED-PAR1Tb. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA). If so desired, optimization to a different organism, such as, *e.g.*, a yeast strain, an insect cell-line or a mammalian cell line, can be done, see, *e.g.*, Steward, *supra*, PCT Patent Serial No. 2005/020578 (Jun. 9, 2005); and Steward, *supra*, PCT Patent Serial No. 2005/XXXXXX (Aug. 3, 2005).

[0212] A similar cloning strategy is used to make pUCBHB1 cloning constructs comprising the polynucleotide molecule of SEQ ID NO: 110 or SEQ ID NO: 137 encoding BoNT/A-ED-PAR1Xa of SEQ ID NO: 86; the polynucleotide molecule of SEQ ID NO: 111 or SEQ ID NO: 138 encoding BoNT/A-ED-PAR2Tp of SEQ ID NO: 87; the polynucleotide molecule of SEQ ID NO: 112 or SEQ ID NO: 139 encoding BoNT/A-ED-PAR2Xa of SEQ ID NO: 88; the polynucleotide molecule of SEQ ID NO: 113 or SEQ ID NO: 140 encoding BoNT/A-ED-PAR3Tb of SEQ ID NO: 89; the polynucleotide molecule of SEQ ID NO: 114 or SEQ ID NO: 141 encoding BoNT/A-ED-PAR3Xa of SEQ ID NO: 90; the polynucleotide molecule of SEQ ID NO: 115 or SEQ ID NO: 142 encoding BoNT/A-ED-PAR4Tb of SEQ ID NO: 91; and the polynucleotide molecule of SEQ ID NO: 116 or SEQ ID NO: 143 encoding BoNT/A-ED-PAR4Xa of SEQ ID NO: 92. In addition, one skilled in the art can modify Clostridial toxins, such as, *e.g.*, BoNT/B,

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BoNT/C1, BoNT/D, BoNT/E, BoNT/F, BoNT/G and TeNT, so that these toxins possess the PAR attributes of the modified BoNT/A described above and make them using similar cloning strategy.

[0213] To construct pET29/BoNT/A-ED-PAR1Tb, a pUCBHB1/BoNT/A-ED-PAR1Tb construct is digested with restriction endonucleases that 1) excise the insert comprising the open reading frame of SEQ ID NO: 136 encoding BoNT/A-ED-PAR1Tb; and 2) enable this insert to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). This insert is subcloned using a T4 DNA ligase procedure into a pET29 vector that is digested with appropriate restriction endonucleases to yield pET29/BoNT/A-ED-PAR1Tb. The ligation mixture is transformed into chemically competent *E. coli* DH5 α cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 50 μ g/mL of Kanamycin, and placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs are identified as Kanamycin resistant colonies. Candidate constructs are isolated using an alkaline lysis plasmid mini-preparation procedure and analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy yielded a pET29 expression construct comprising the polynucleotide molecule of SEQ ID NO: 136 encoding the BoNT/A-ED-PAR1Tb of SEQ ID NO: 85 operably-linked to a carboxyl terminal polyhistidine affinity binding peptide (FIG. 7).

[0214] A similar cloning strategy is used to make pET29 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 109 encoding BoNT/A-ED-PAR1Tb of SEQ ID NO: 85; SEQ ID NO: 110 or SEQ ID NO: 137 encoding BoNT/A-ED-PAR1Xa of SEQ ID NO: 86; the polynucleotide molecule of SEQ ID NO: 111 or SEQ ID NO: 138 encoding BoNT/A-ED-PAR2Tp of SEQ ID NO: 87; the polynucleotide molecule of SEQ ID NO: 112 or SEQ ID NO: 139 encoding BoNT/A-ED-PAR2Xa of SEQ ID NO: 88; the polynucleotide molecule of SEQ ID NO: 113 or SEQ ID NO: 140 encoding BoNT/A-ED-PAR3Tb of SEQ ID NO: 89; the polynucleotide molecule of SEQ ID NO: 114 or SEQ ID NO: 141 encoding BoNT/A-ED-PAR3Xa of SEQ ID NO: 90; the polynucleotide molecule of SEQ ID NO: 115 or SEQ ID NO: 142 encoding BoNT/A-ED-PAR4Tb of SEQ ID NO: 91; and the polynucleotide molecule of SEQ ID NO: 116 or SEQ ID NO: 143 encoding BoNT/A-ED-PAR4Xa of SEQ ID NO: 92.

Example 2

Construction of BoNT/A-TD-PAR1Tb

[0215] This example illustrates how to make a modified Clostridial toxin comprising a PAR binding domain located at the amino terminus of the heavy chain region comprising the translocation domain.

[0216] A polynucleotide molecule (SEQ ID NO: 117) based on BoNT/A-TD-PAR1Tb (SEQ ID NO: 93) is synthesized using standard procedures (BlueHeron® Biotechnology, Bothell, WA). Oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length

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polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-TD-PAR1Tb. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA).

[0217] If desired, an expression optimized polynucleotide molecule (SEQ ID NO: 144) based on BoNT/A-TD-PAR1Tb (SEQ ID NO: 93) can be synthesized in order to improve expression in an *Escherichia coli* strain. The open reading frame comprising the polynucleotide molecule is optimized to improve expression in an *Escherichia coli* strain. The polynucleotide molecule encoding the BoNT/A-TD-PAR1Tb can be modified to 1) contain synonymous codons typically present in native polynucleotide molecules of an *Escherichia coli* strain; 2) contain a G+C content that more closely matches the average G+C content of native polynucleotide molecules found in an *Escherichia coli* strain; 3) reduce polynucleotide regions found within the polynucleotide molecule; and/or 4) eliminate internal regulatory or structural sites found within the polynucleotide molecule, see, *e.g.*, Lance E. Steward *et al.* Optimizing Expression of Active Botulinum Toxin Type E, PCT Patent Serial No. 2005/020578 (Jun. 9, 2005); Lance E. Steward *et al.* Optimizing Expression of Active Botulinum Toxin Type A, PCT Patent Serial No. 2005/XXXXXX (Aug. 3, 2005). Once sequence optimization is complete, oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate BoNT/A-TD-PAR1Tb. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA). Is so desired, optimization of the polynucleotide molecule encoding a BoNT/A-TD-PAR1Tb need not be performed, or optimization to a different organism, such as, *e.g.*, a yeast strain, an insect cell-line or a mammalian cell line, can be done instead, see, *e.g.*, Steward, *supra*, PCT Patent Serial No. 2005/020578 (Jun. 9, 2005); and Steward, *supra*, PCT Patent Serial No. 2005/XXXXXX (Aug. 3, 2005).

[0218] A similar cloning strategy is used to make pUCBHB1 cloning constructs comprising the polynucleotide molecule of SEQ ID NO: 118 or SEQ ID NO: 145 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 94; the polynucleotide molecule of SEQ ID NO: 119 or SEQ ID NO: 146 encoding BoNT/A-TD-PAR2Tp of SEQ ID NO: 95; the polynucleotide molecule of SEQ ID NO: 120 or SEQ ID NO: 147 encoding BoNT/A-TD-PAR2Xa of SEQ ID NO: 96; the polynucleotide molecule of SEQ ID NO: 121 or SEQ ID NO: 148 encoding BoNT/A-TD-PAR1Tb of SEQ ID NO: 97; the polynucleotide molecule of SEQ ID NO: 122 or SEQ ID NO: 149 encoding BoNT/A-TD-PAR3Xa of SEQ ID NO: 98; the polynucleotide molecule of SEQ ID NO: 123 or SEQ ID NO: 150 encoding BoNT/A-TD-PAR4Tb of SEQ ID NO: 99; and the polynucleotide molecule of SEQ ID NO: 124 or SEQ ID NO: 151 encoding BoNT/A-TD-PAR4Xa of SEQ ID NO: 100. In addition, one skilled in the art can modify Clostridial toxins, such as, *e.g.*, BoNT/B,

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BoNT/C1, BoNT/D, BoNT/E, BoNT/F, BoNT/G and TeNT, so that these toxins possess the PAR attributes of the modified BoNT/A described above and make them using similar cloning strategy.

[0219] To construct pET29/BoNT/A-TD-PAR1Tb, a pUCBHB1/BoNT/A-TD-PAR1Tb construct is digested with restriction endonucleases that 1) excise the insert comprising the open reading frame of SEQ ID NO: 144 encoding BoNT/A-TD-PAR1Tb; and 2) enable this insert to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). This insert is subcloned using a T4 DNA ligase procedure into a pET29 vector that is digested with appropriate restriction endonucleases to yield pET29/BoNT/A-TD-PAR1Tb. The ligation mixture is transformed into chemically competent *E. coli* DH5 α cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 50 μ g/mL of Kanamycin, and placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs are identified as Kanamycin resistant colonies. Candidate constructs are isolated using an alkaline lysis plasmid mini-preparation procedure and analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy yielded a pET29 expression construct comprising the polynucleotide molecule of SEQ ID NO: 144 encoding the BoNT/A-TD-PAR1Tb of SEQ ID NO: 93 operably-linked to a carboxyl terminal polyhistidine affinity binding peptide (FIG. 8).

[0220] A similar cloning strategy is used to make pET29 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 117 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 93; SEQ ID NO: 118 or SEQ ID NO: 145 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 94; the polynucleotide molecule of SEQ ID NO: 119 or SEQ ID NO: 146 encoding BoNT/A-TD-PAR2Tp of SEQ ID NO: 95; the polynucleotide molecule of SEQ ID NO: 120 or SEQ ID NO: 147 encoding BoNT/A-TD-PAR2Xa of SEQ ID NO: 96; the polynucleotide molecule of SEQ ID NO: 121 or SEQ ID NO: 148 encoding BoNT/A-TD-PAR1Tb of SEQ ID NO: 97; the polynucleotide molecule of SEQ ID NO: 122 or SEQ ID NO: 149 encoding BoNT/A-TD-PAR3Xa of SEQ ID NO: 98; the polynucleotide molecule of SEQ ID NO: 123 or SEQ ID NO: 150 encoding BoNT/A-TD-PAR4Tb of SEQ ID NO: 99; and the polynucleotide molecule of SEQ ID NO: 124 or SEQ ID NO: 151 encoding BoNT/A-TD-PAR4Xa of SEQ ID NO: 100.

Example 3

Construction of BoNT/A-BD-PAR1Tb

[0221] This example illustrates how to make a modified Clostridial toxin comprising a PAR binding domain located at the amino terminus of the heavy chain region comprising the binding domain.

[0222] A polynucleotide molecule (SEQ ID NO: 125) based on BoNT/A-BD-PAR1Tb (SEQ ID NO: 101) is synthesized using standard procedures (BlueHeron® Biotechnology, Bothell, WA). Oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the

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full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate pUCBHB1/BoNT/A-BD-PAR1Tb. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA).

[0223] If desired, an expression optimized polynucleotide molecule (SEQ ID NO: 152) based on BoNT/A-BD-PAR1Tb (SEQ ID NO: 101) can be synthesized in order to improve expression in an *Escherichia coli* strain. The open reading frame comprising the polynucleotide molecule is optimized to improve expression in an *Escherichia coli* strain. The polynucleotide molecule encoding the BoNT/A-BD-PAR1Tb can be modified to 1) contain synonymous codons typically present in native polynucleotide molecules of an *Escherichia coli* strain; 2) contain a G+C content that more closely matches the average G+C content of native polynucleotide molecules found in an *Escherichia coli* strain; 3) reduce polymononucleotide regions found within the polynucleotide molecule; and/or 4) eliminate internal regulatory or structural sites found within the polynucleotide molecule, see, *e.g.*, Lance E. Steward *et al.* Optimizing Expression of Active Botulinum Toxin Type E, PCT Patent Serial No. 2005/020578 (Jun. 9, 2005); Lance E. Steward *et al.* Optimizing Expression of Active Botulinum Toxin Type A, PCT Patent Serial No. 2005/XXXXXX (Aug. 3, 2005). Once sequence optimization is complete, oligonucleotides of 20 to 50 bases in length are synthesized using standard phosphoramidite synthesis. These oligonucleotides are hybridized into double stranded duplexes that are ligated together to assemble the full-length polynucleotide molecule. This polynucleotide molecule is cloned using standard molecular biology methods into a pUCBHB1 vector at the *Sma*I site to generate BoNT/A-BD-PAR1Tb. The synthesized polynucleotide molecule is verified by sequencing using Big Dye Terminator™ Chemistry 3.1 (Applied Biosystems, Foster City, CA) and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA). If so desired, optimization of the polynucleotide molecule encoding a BoNT/A-BD-PAR1Tb need not be performed, or optimization to a different organism, such as, *e.g.*, a yeast strain, an insect cell-line or a mammalian cell line, can be done instead, see, *e.g.*, Steward, *supra*, PCT Patent Serial No. 2005/020578 (Jun. 9, 2005); and Steward, *supra*, PCT Patent Serial No. 2005/XXXXXX (Aug. 3, 2005).

[0224] A similar cloning strategy is used to make pUCBHB1 cloning constructs comprising the polynucleotide molecule of SEQ ID NO: 126 or SEQ ID NO: 153 encoding BoNT/A-BD-PAR1Xa of SEQ ID NO: 102; the polynucleotide molecule of SEQ ID NO: 127 or SEQ ID NO: 154 encoding BoNT/A-BD-PAR2Tp of SEQ ID NO: 103; the polynucleotide molecule of SEQ ID NO: 128 or SEQ ID NO: 155 encoding BoNT/A-BD-PAR2Xa of SEQ ID NO: 104; the polynucleotide molecule of SEQ ID NO: 129 or SEQ ID NO: 156 encoding BoNT/A-BD-PAR3Tb of SEQ ID NO: 105; the polynucleotide molecule of SEQ ID NO: 130 or SEQ ID NO: 157 encoding BoNT/A-BD-PAR3Xa of SEQ ID NO: 106; the polynucleotide molecule of SEQ ID NO: 131 or SEQ ID NO: 158 encoding BoNT/A-BD-PAR4Tb of SEQ ID NO: 107; and the polynucleotide molecule of SEQ ID NO: 132 or SEQ ID NO: 159 encoding BoNT/A-BD-PAR4Xa of SEQ ID NO: 108. In addition, one skilled in the art can modify Clostridial toxins, such as, *e.g.*, BoNT/B,

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BoNT/C1, BoNT/D, BoNT/E, BoNT/F, BoNT/G and TeNT, so that these toxins possess the PAR attributes of the modified BoNT/A described above and make them using similar cloning strategy.

[0225] To construct pET29/BoNT/A-BD-PAR1Tb, a pUCBHB1/BoNT/A-BD-PAR1Tb construct is digested with restriction endonucleases that 1) excise the insert comprising the open reading frame of SEQ ID NO: 152 encoding BoNT/A-BD-PAR1Tb; and 2) enable this insert to be operably-linked to a pET29 vector (EMD Biosciences-Novagen, Madison, WI). This insert is subcloned using a T4 DNA ligase procedure into a pET29 vector that is digested with appropriate restriction endonucleases to yield pET29/BoNT/A-BD-PAR1Tb. The ligation mixture is transformed into chemically competent *E. coli* DH5 α cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 50 μ g/mL of Kanamycin, and placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs are identified as Kanamycin resistant colonies. Candidate constructs are isolated using an alkaline lysis plasmid mini-preparation procedure and analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy yielded a pET29 expression construct comprising the polynucleotide molecule of SEQ ID NO: 152 encoding the BoNT/A-BD-PAR1Tb of SEQ ID NO: 101 operably-linked to a carboxyl terminal polyhistidine affinity binding peptide (FIG. 9).

[0226] A similar cloning strategy is used to make pET29 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 125 encoding BoNT/A-BD-PAR1Tb of SEQ ID NO: 101; the polynucleotide molecule of SEQ ID NO: 126 or SEQ ID NO: 153 encoding BoNT/A-BD-PAR1Xa of SEQ ID NO: 102; the polynucleotide molecule of SEQ ID NO: 127 or SEQ ID NO: 154 encoding BoNT/A-BD-PAR2Tp of SEQ ID NO: 103; the polynucleotide molecule of SEQ ID NO: 128 or SEQ ID NO: 155 encoding BoNT/A-BD-PAR2Xa of SEQ ID NO: 104; the polynucleotide molecule of SEQ ID NO: 129 or SEQ ID NO: 156 encoding BoNT/A-BD-PAR3Tb of SEQ ID NO: 105; the polynucleotide molecule of SEQ ID NO: 130 or SEQ ID NO: 157 encoding BoNT/A-BD-PAR3Xa of SEQ ID NO: 106; the polynucleotide molecule of SEQ ID NO: 131 or SEQ ID NO: 158 encoding BoNT/A-BD-PAR4Tb of SEQ ID NO: 107; and the polynucleotide molecule of SEQ ID NO: 132 or SEQ ID NO: 159 encoding BoNT/A-BD-PAR4Xa of SEQ ID NO: 108.

Example 4

Expression of Modified Clostridial Toxins in a Bacterial Cell

[0227] The following example illustrates a procedure useful for expressing any of the modified Clostridial toxins disclosed in the present specification in a bacterial cell.

[0228] An expression construct, such as, *e.g.*, pET29/BoNT/A-ED-PAR1Tb, pET29/BoNT/A-TD-PAR1Tb or pET29/BoNT/A-BD-PAR1Tb, see, *e.g.*, Examples 1, 2 and 3, is introduced into chemically competent *E. coli* BL21 (DE3) cells (Invitrogen, Inc, Carlsbad, CA) using a heat-shock transformation protocol. The

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heat-shock reaction is plated onto 1.5% Luria-Bertani agar plates (pH 7.0) containing 50 µg/mL of Kanamycin and is placed in a 37 °C incubator for overnight growth. Kanamycin-resistant colonies of transformed *E. coli* containing the expression construct, such as, *e.g.*, pET29/BoNT/A-ED-PAR1Tb, pET29/BoNT/A-TD-PAR1Tb or pET29/BoNT/A-BD-PAR1Tb, are used to inoculate a baffled flask containing 3.0 mL of PA-0.5G media containing 50 µg/mL of Kanamycin which is then placed in a 37 °C incubator, shaking at 250 rpm, for overnight growth. The resulting overnight starter culture is in turn used to inoculate a 3 L baffled flask containing ZYP-5052 autoinducing media containing 50 µg/mL of Kanamycin at a dilution of 1:1000. Culture volumes ranged from about 600 mL (20% flask volume) to about 750 mL (25% flask volume). These cultures are grown in a 37 °C incubator shaking at 250 rpm for approximately 5.5 hours and are then transferred to a 16 °C incubator shaking at 250 rpm for overnight expression. Cells are harvested by centrifugation (4,000 rpm at 4 °C for 20-30 minutes) and are used immediately, or stored dry at -80 °C until needed.

Example 5

Purification and Quantification of Modified Clostridial Toxins

[0229] The following example illustrates methods useful for purification and quantification of any modified Clostridial toxins disclosed in the present specification.

[0230] For immobilized metal affinity chromatography (IMAC) protein purification, *E. coli* BL21 (DE3) cell pellets used to express a modified Clostridial toxin, as described in Example 4, are resuspended in Column Binding Buffer (25 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 7.8; 500 mM sodium chloride; 10 mM imidazole; 2x Protease Inhibitor Cocktail Set III (EMD Biosciences-Calbiochem, San Diego CA); 5 units/mL of Benzonase (EMD Biosciences-Novagen, Madison, WI); 0.1% (v/v) Triton-X[®] 100, 4-octylphenol polyethoxylate; 10% (v/v) glycerol), and then are transferred to a cold Oakridge centrifuge tube. The cell suspension is sonicated on ice (10-12 pulses of 10 seconds at 40% amplitude with 60 seconds cooling intervals on a Branson Digital Sonifier) in order to lyse the cells and then is centrifuged (16,000 rpm at 4 °C for 20 minutes) to clarify the lysate. An immobilized metal affinity chromatography column is prepared using a 20 mL Econo-Pac column support (Bio-Rad Laboratories, Hercules, CA) packed with 2.5-5.0 mL of TALON[™] SuperFlow Co²⁺ affinity resin (BD Biosciences-Clontech, Palo Alto, CA), which is then equilibrated by rinsing with 5 column volumes of deionized, distilled water, followed by 5 column volumes of Column Binding Buffer. The clarified lysate is applied slowly to the equilibrated column by gravity flow (approximately 0.25-0.3 mL/minute). The column is then washed with 5 column volumes of Column Wash Buffer (*N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 7.8; 500 mM sodium chloride; 10 mM imidazole; 0.1% (v/v) Triton-X[®] 100, 4-octylphenol polyethoxylate; 10% (v/v) glycerol). The Clostridial toxin is eluted with 20-30 mL of Column Elution Buffer (25 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 7.8; 500 mM sodium chloride; 500 mM imidazole; 0.1% (v/v) Triton-X[®] 100, 4-octylphenol polyethoxylate; 10% (v/v) glycerol) and is collected in approximately twelve 1 mL fractions. The amount of Clostridial toxin

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contained in each elution fraction is determined by a Bradford dye assay. In this procedure, 20 μ L aliquots of each 1.0 mL fraction is combined with 200 μ L of Bio-Rad Protein Reagent (Bio-Rad Laboratories, Hercules, CA), diluted 1 to 4 with deionized, distilled water, and then the intensity of the colorimetric signal is measured using a spectrophotometer. The five fractions with the strongest signal are considered the elution peak and are combined together. Total protein yield is determined by estimating the total protein concentration of the pooled peak elution fractions using bovine gamma globulin as a standard (Bio-Rad Laboratories, Hercules, CA).

[0231] For purification of a modified Clostridial toxin using a FPLC desalting column, a HiPrep™ 26/10 size exclusion column (Amersham Biosciences, Piscataway, NJ) is pre-equilibrated with 80 mL of 4 °C Column Buffer (50 mM sodium phosphate, pH 6.5). After the column is equilibrated, a Clostridial toxin sample is applied to the size exclusion column with an isocratic mobile phase of 4 °C Column Buffer and at a flow rate of 10 mL/minute using a BioLogic DuoFlow chromatography system (Bio-Rad Laboratories, Hercules, CA). The desalted modified Clostridial toxin sample is collected as a single fraction of approximately 7-12 mL.

[0232] For purification of a modified Clostridial toxin using a FPLC ion exchange column, a Clostridial toxin sample that has been desalted following elution from an IMAC column is applied to a 1 mL Q1™ anion exchange column (Bio-Rad Laboratories, Hercules, CA) using a BioLogic DuoFlow chromatography system (Bio-Rad Laboratories, Hercules, CA). The sample is applied to the column in 4 °C Column Buffer (50 mM sodium phosphate, pH 6.5) and is eluted by linear gradient with 4 °C Elution Buffer (50 mM sodium phosphate, 1 M sodium chloride, pH 6.5) as follows: step 1, 5.0 mL of 5% Elution Buffer at a flow rate of 1 mL/minute; step 2, 20.0 mL of 5-30% Elution Buffer at a flow rate of 1 mL/minute; step 3, 2.0 mL of 50% Elution Buffer at a flow rate of 1.0 mL/minute; step 4, 4.0 mL of 100% Elution Buffer at a flow rate of 1.0 mL/minute; and step 5, 5.0 mL of 0% Elution Buffer at a flow rate of 1.0 mL/minute. Elution of Clostridial toxin from the column is monitored at 280, 260, and 214 nm, and peaks absorbing above a minimum threshold (0.01 au) at 280 nm are collected. Most of the Clostridial toxin will elute at a sodium chloride concentration of approximately 100 to 200 mM. Average total yields of Clostridial toxin will be determined by a Bradford assay.

[0233] Expression of a modified Clostridial toxin is analyzed by polyacrylamide gel electrophoresis. Samples purified using the procedure described above are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and are separated by MOPS polyacrylamide gel electrophoresis using NuPAGE® Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen, Inc, Carlsbad, CA) under denaturing, reducing conditions. Gels are stained with SYPRO® Ruby (Bio-Rad Laboratories, Hercules, CA) and the separated polypeptides are imaged using a Fluor-S MAX Multimager (Bio-Rad Laboratories, Hercules, CA) for quantification of Clostridial toxin expression levels. The size and amount of the Clostridial toxin is determined by comparison to MagicMark™ protein molecular weight standards (Invitrogen, Inc, Carlsbad, CA).

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[0234] Expression of modified Clostridial toxin is also analyzed by Western blot analysis. Protein samples purified using the procedure described above are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and are separated by MOPS polyacrylamide gel electrophoresis using NuPAGE® Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen, Inc, Carlsbad, CA) under denaturing, reducing conditions. Separated polypeptides are transferred from the gel onto polyvinylidene fluoride (PVDF) membranes (Invitrogen, Inc, Carlsbad, CA) by Western blotting using a Trans-Blot® SD semi-dry electrophoretic transfer cell apparatus (Bio-Rad Laboratories, Hercules, CA). PVDF membranes are blocked by incubating at room temperature for 2 hours in a solution containing 25 mM Tris-Buffered Saline (25 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloric acid (Tris-HCl)(pH 7.4), 137 mM sodium chloride, 2.7 mM potassium chloride), 0.1% TWEEN-20®, polyoxyethylene (20) sorbitan monolaureate, 2% bovine serum albumin, 5% nonfat dry milk. Blocked membranes are incubated at 4 °C for overnight in Tris-Buffered Saline TWEEN-20® (25 mM Tris-Buffered Saline, 0.1% TWEEN-20®, polyoxyethylene (20) sorbitan monolaureate) containing appropriate primary antibodies as a probe. Primary antibody probed blots are washed three times for 15 minutes each time in Tris-Buffered Saline TWEEN-20®. Washed membranes are incubated at room temperature for 2 hours in Tris-Buffered Saline TWEEN-20® containing an appropriate immunoglobulin G antibody conjugated to horseradish peroxidase as a secondary antibody. Secondary antibody-probed blots are washed three times for 15 minutes each time in Tris-Buffered Saline TWEEN-20®. Signal detection of the labeled Clostridial toxin are visualized using the ECL Plus™ Western Blot Detection System (Amersham Biosciences, Piscataway, NJ) and are imaged with a Typhoon 9410 Variable Mode Imager (Amersham Biosciences, Piscataway, NJ) for quantification of modified Clostridial toxin expression levels.

Example 6

Expression of Modified Clostridial Toxins in a Yeast Cell

[0235] The following example illustrates a procedure useful for expressing any of the modified Clostridial toxins disclosed in the present specification in a yeast cell.

[0236] To construct a suitable yeast expression construct encoding a modified Clostridial toxin, restriction endonuclease sites suitable for cloning an operably linked polynucleotide molecule into a pPIC A vector (Invitrogen, Inc, Carlsbad, CA) are incorporated into the 5'- and 3' ends of the polynucleotide molecule SEQ ID NO: 136 encoding BoNT/A-ED-PAR1Tb of SEQ ID NO: 85. This polynucleotide molecule is synthesized and a pUCBHB1/BoNT/A-ED-PAR1Tb construct is obtained as described in Example 1. This construct is digested with restriction enzymes that 1) excise the insert containing the open reading frame of SEQ ID NO: 136 encoding BoNT/A-ED-PAR1Tb; and 2) enable this insert to be operably-linked to a pPIC A vector. This insert is subcloned using a T4 DNA ligase procedure into a pPIC A vector that is digested with appropriate restriction endonucleases to yield pPIC A/BoNT/A-ED-PAR1Tb. The ligation mixture is transformed into chemically competent *E. coli* DH5α cells (Invitrogen, Inc,

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Carlsbad, CA) using a heat shock method, plated on 1.5% low salt Luria-Bertani agar plates (pH 7.5) containing 25 µg/mL of Zeocin™, and placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs are identified as Zeocin™ resistant colonies. Candidate constructs are isolated using an alkaline lysis plasmid mini-preparation procedure and analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy yielded a pPIC A expression construct comprising the polynucleotide molecule of SEQ ID NO: 136 encoding the BoNT/A-ED-PAR1Tb of SEQ ID NO: 85 operably-linked to a carboxyl-terminal c-myc and polyhistidine binding peptides (FIG. 10).

[0237] A similar cloning strategy is used to make pPIC A expression constructs comprising the polynucleotide molecule of SEQ ID NO: 109 encoding BoNT/A-ED-PAR1Tb of SEQ ID NO: 85; SEQ ID NO: 110 or SEQ ID NO: 137 encoding BoNT/A-ED-PAR1Xa of SEQ ID NO: 86; the polynucleotide molecule of SEQ ID NO: 111 or SEQ ID NO: 138 encoding BoNT/A-ED-PAR2Tp of SEQ ID NO: 87; the polynucleotide molecule of SEQ ID NO: 112 or SEQ ID NO: 139 encoding BoNT/A-ED-PAR2Xa of SEQ ID NO: 88; the polynucleotide molecule of SEQ ID NO: 113 or SEQ ID NO: 140 encoding BoNT/A-ED-PAR3Tb of SEQ ID NO: 89; the polynucleotide molecule of SEQ ID NO: 114 or SEQ ID NO: 141 encoding BoNT/A-ED-PAR3Xa of SEQ ID NO: 90; the polynucleotide molecule of SEQ ID NO: 115 or SEQ ID NO: 142 encoding BoNT/A-ED-PAR4Tb of SEQ ID NO: 91; and the polynucleotide molecule of SEQ ID NO: 116 or SEQ ID NO: 143 encoding BoNT/A-ED-PAR4Xa of SEQ ID NO: 92.

[0238] A similar cloning strategy is used to make pPIC A expression constructs comprising the polynucleotide molecule of SEQ ID NO: 117 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 93; SEQ ID NO: 118 or SEQ ID NO: 145 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 94; the polynucleotide molecule of SEQ ID NO: 119 or SEQ ID NO: 146 encoding BoNT/A-TD-PAR2Tp of SEQ ID NO: 95; the polynucleotide molecule of SEQ ID NO: 120 or SEQ ID NO: 147 encoding BoNT/A-TD-PAR2Xa of SEQ ID NO: 96; the polynucleotide molecule of SEQ ID NO: 121 or SEQ ID NO: 148 encoding BoNT/A-TD-PAR1Tb of SEQ ID NO: 97; the polynucleotide molecule of SEQ ID NO: 122 or SEQ ID NO: 149 encoding BoNT/A-TD-PAR3Xa of SEQ ID NO: 98; the polynucleotide molecule of SEQ ID NO: 123 or SEQ ID NO: 150 encoding BoNT/A-TD-PAR4Tb of SEQ ID NO: 99; and the polynucleotide molecule of SEQ ID NO: 124 or SEQ ID NO: 151 encoding BoNT/A-TD-PAR4Xa of SEQ ID NO: 100.

[0239] A similar cloning strategy is used to make pPIC A expression constructs comprising the polynucleotide molecule of SEQ ID NO: 125 encoding BoNT/A-BD-PAR1Tb of SEQ ID NO: 101; the polynucleotide molecule of SEQ ID NO: 126 or SEQ ID NO: 153 encoding BoNT/A-BD-PAR1Xa of SEQ ID NO: 102; the polynucleotide molecule of SEQ ID NO: 127 or SEQ ID NO: 154 encoding BoNT/A-BD-PAR2Tp of SEQ ID NO: 103; the polynucleotide molecule of SEQ ID NO: 128 or SEQ ID NO: 155 encoding BoNT/A-BD-PAR2Xa of SEQ ID NO: 104; the polynucleotide molecule of SEQ ID NO: 129 or SEQ ID NO: 156 encoding BoNT/A-BD-PAR3Tb of SEQ ID NO: 105; the polynucleotide molecule of SEQ ID NO: 130 or SEQ ID NO: 157 encoding BoNT/A-BD-PAR3Xa of SEQ ID NO: 106; the polynucleotide

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molecule of SEQ ID NO: 131 or SEQ ID NO: 158 encoding BoNT/A-BD-PAR4Tb of SEQ ID NO: 107; and the polynucleotide molecule of SEQ ID NO: 132 or SEQ ID NO: 159 encoding BoNT/A-BD-PAR4Xa of SEQ ID NO: 108.

[0240] To construct a yeast cell line expressing a modified Clostridial toxin, pPICZ A/BoNT/A-ED-PAR1Tb is digested with a suitable restriction endonuclease (*i.e.*, *SacI*, *PmeI* or *BsfXI*) and the resulting linearized expression construct is transformed into an appropriate *P. pastoris* Mut^S strain KM71H using an electroporation method. The transformation mixture is plated on 1.5% YPDS agar plates (pH 7.5) containing 100 µg/mL of ZeocinTM and placed in a 28-30 °C incubator for 1-3 days of growth. Selection of transformants integrating the pPICZ A/BoNT/A-ED-PAR1Tb at the 5' AOX1 locus is determined by colony resistance to ZeocinTM. A similar strategy is used to make a cell line containing a pPICZ A expression construct containing SEQ ID NO: 2 used as a control for expression levels. Cell lines integrating a pPICZ A/BoNT/A-ED-PAR1Tb construct is tested for BoNT/A-ED-PAR1Tb expression using a small-scale expression test. Isolated colonies from test cell lines that have integrated pPICZ A/BoNT/A-ED-PAR1Tb are used to inoculate 1.0 L baffled flasks containing 100 mL of MGYH media and grown at about 28-30 °C in a shaker incubator (250 rpm) until the culture reaches an OD₆₀₀=2-6 (approximately 16-18 hours). Cells are harvested by centrifugation (3,000x *g* at 22 °C for 5 minutes). To induce expression, the cell pellet is resuspended in 15 mL of MMH media and 100% methanol is added to a final concentration of 0.5%. Cultures are grown at about 28-30 °C in a shaker incubator (250 rpm) for six days. Additional 100% methanol is added to the culture every 24 hours to a final concentration of 0.5%. A 1.0 mL test aliquot is taken from the culture every 24 hours starting at time zero and ending at time 144 hours. Cells are harvested from the aliquots by microcentrifugation to pellet the cells and lysed using three freeze-thaw rounds consisting of -80 °C for 5 minutes, then 37 °C for 5 minutes. Lysis samples are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and expression from established cell lines is measured by Western blot analysis (as described in Example 5) using either anti-BoNT/A, anti-myc or anti-His antibodies in order to identify lines expressing BoNT/A-ED-PAR1Tb. The *P. pastoris* Mut^S KM71H cell line showing the highest expression level of BoNT/A-ED-PAR1Tb is selected for large-scale expression using commercial fermentation procedures. Procedures for large-scale expression are as outlined above except the culture volume is approximately 2.5 L MGYH media grown in a 5 L BioFlo 3000 fermentor and concentrations of all reagents will be proportionally increased for this volume.

[0241] BoNT/A-ED-PAR1Tb is purified using the IMAC procedure, as described in Example 5. Expression from each culture is evaluated by a Bradford dye assay, polyacrylamide gel electrophoresis and Western blot analysis (as described in Example 5) in order to determine whether the amounts of BoNT/A-ED-PAR1Tb produced.

Example 7

Expression of Modified Clostridial Toxins in an Insect Cell

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[0242] The following example illustrates a procedure useful for expressing any of the modified Clostridial toxins disclosed in the present specification in an insect cell.

[0243] To construct suitable an insect expression construct encoding a modified Clostridial toxin, restriction endonuclease sites suitable for cloning an operably linked polynucleotide molecule into a pBACgus3 vector (EMD Biosciences-Novagen, Madison, WI) are incorporated into the 5'- and 3' ends of the polynucleotide molecule SEQ ID NO: 136 encoding BoNT/A-ED-PAR1Tb of SEQ ID NO: 85. This polynucleotide molecule is synthesized and a pUCBHB1/BoNT/A-ED-PAR1Tb construct is obtained as described in Example 1. This construct is digested with restriction enzymes that 1) excise the insert containing the open reading frame of SEQ ID NO: 136 encoding BoNT/A-ED-PAR1Tb; and 2) enable this insert to be operably-linked to a pBACgus3 vector. This insert is subcloned using a T4 DNA ligase procedure into a pBACgus3 vector that is digested with appropriate restriction endonucleases to yield pBACgus3/BoNT/A-ED-PAR1Tb. The ligation mixture is transformed into chemically competent *E. coli* DH5 α cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 100 μ g/mL of Ampicillin, and placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs are identified as Ampicillin resistant colonies. Candidate constructs are isolated using an alkaline lysis plasmid mini-preparation procedure and analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy yielded a pBACgus3 expression construct comprising the polynucleotide molecule of SEQ ID NO: 136 encoding the BoNT/A-ED-PAR1Tb of SEQ ID NO: 85 operably linked to an amino-terminal gp64 signal peptide and a carboxyl-terminal, Thrombin cleavable, polyhistidine affinity binding peptide (FIG. 11).

[0244] A similar cloning strategy is used to make pBACgus3 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 109 encoding BoNT/A-ED-PAR1Tb of SEQ ID NO: 85; SEQ ID NO: 110 or SEQ ID NO: 137 encoding BoNT/A-ED-PAR1Xa of SEQ ID NO: 86; the polynucleotide molecule of SEQ ID NO: 111 or SEQ ID NO: 138 encoding BoNT/A-ED-PAR2Tp of SEQ ID NO: 87; the polynucleotide molecule of SEQ ID NO: 112 or SEQ ID NO: 139 encoding BoNT/A-ED-PAR2Xa of SEQ ID NO: 88; the polynucleotide molecule of SEQ ID NO: 113 or SEQ ID NO: 140 encoding BoNT/A-ED-PAR3Tb of SEQ ID NO: 89; the polynucleotide molecule of SEQ ID NO: 114 or SEQ ID NO: 141 encoding BoNT/A-ED-PAR3Xa of SEQ ID NO: 90; the polynucleotide molecule of SEQ ID NO: 115 or SEQ ID NO: 142 encoding BoNT/A-ED-PAR4Tb of SEQ ID NO: 91; and the polynucleotide molecule of SEQ ID NO: 116 or SEQ ID NO: 143 encoding BoNT/A-ED-PAR4Xa of SEQ ID NO: 92.

[0245] A similar cloning strategy is used to make pBACgus3 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 117 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 93; SEQ ID NO: 118 or SEQ ID NO: 145 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 94; the polynucleotide molecule of SEQ ID NO: 119 or SEQ ID NO: 146 encoding BoNT/A-TD-PAR2Tp of SEQ ID NO: 95; the polynucleotide molecule of SEQ ID NO: 120 or SEQ ID NO: 147 encoding BoNT/A-TD-PAR2Xa of SEQ

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ID NO: 96; the polynucleotide molecule of SEQ ID NO: 121 or SEQ ID NO: 148 encoding BoNT/A-TD-PAR1Tb of SEQ ID NO: 97; the polynucleotide molecule of SEQ ID NO: 122 or SEQ ID NO: 149 encoding BoNT/A-TD-PAR3Xa of SEQ ID NO: 98; the polynucleotide molecule of SEQ ID NO: 123 or SEQ ID NO: 150 encoding BoNT/A-TD-PAR4Tb of SEQ ID NO: 99; and the polynucleotide molecule of SEQ ID NO: 124 or SEQ ID NO: 151 encoding BoNT/A-TD-PAR4Xa of SEQ ID NO: 100.

[0246] A similar cloning strategy is used to make pBACgus3 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 125 encoding BoNT/A-BD-PAR1Tb of SEQ ID NO: 101; the polynucleotide molecule of SEQ ID NO: 126 or SEQ ID NO: 153 encoding BoNT/A-BD-PAR1Xa of SEQ ID NO: 102; the polynucleotide molecule of SEQ ID NO: 127 or SEQ ID NO: 154 encoding BoNT/A-BD-PAR2Tp of SEQ ID NO: 103; the polynucleotide molecule of SEQ ID NO: 128 or SEQ ID NO: 155 encoding BoNT/A-BD-PAR2Xa of SEQ ID NO: 104; the polynucleotide molecule of SEQ ID NO: 129 or SEQ ID NO: 156 encoding BoNT/A-BD-PAR3Tb of SEQ ID NO: 105; the polynucleotide molecule of SEQ ID NO: 130 or SEQ ID NO: 157 encoding BoNT/A-BD-PAR3Xa of SEQ ID NO: 106; the polynucleotide molecule of SEQ ID NO: 131 or SEQ ID NO: 158 encoding BoNT/A-BD-PAR4Tb of SEQ ID NO: 107; and the polynucleotide molecule of SEQ ID NO: 132 or SEQ ID NO: 159 encoding BoNT/A-BD-PAR4Xa of SEQ ID NO: 108.

[0247] To express a modified Clostridial toxin using a baculoviral expression system, about 2.5×10^6 Sf9 cells are plated in four 60 mm culture dishes containing 2 mL of BacVector[®] Insect media (EMD Biosciences-Novagen, Madison, WI) and incubated for approximately 20 minutes in a 28 °C incubator. For each transfection, a 50 µL transfection solution is prepared in a 6 mL polystyrene tube by adding 25 µL of BacVector[®] Insect media containing 100 ng of a pBACgus3 construct encoding a modified Clostridial toxin, such as, *e.g.*, pBACgus3/BoNT/A-ED-PAR1Tb, and 500 ng TlowE transfer plasmid to 25 µL of diluted Insect GeneJuice[®] containing 5 µL Insect GeneJuice[®] (EMD Biosciences-Novagen, Madison, WI) and 20 µL nuclease-free water and this solution is incubated for approximately 15 minutes. After the 15 minute incubation, add 450 µL BacVector[®] media to the transfection solution and mix gently. Using this stock transfection solution as the 1/10 dilution make additional transfection solutions of 1/50, 1/250 and 1/1250 dilutions. Add 100 µL of a transfection solution to the Sf9 cells from one of the four 60 mm culture dishes, twice washed with antibiotic-free, serum-free BacVector[®] Insect media and incubate at 22 °C. After one hour, add 6 mL of 1% BacPlaque agarose-BacVector[®] Insect media containing 5% bovine serum albumin. After the agarose is solidified, add 2 mL BacVector[®] Insect media containing 5% bovine serum albumin to the transfected cells and transfer the cells to a 28 °C incubator for 3-5 days until plaques are visible. After 3-5 days post-transfection, plaques in the monolayer will be stained for β-glucuronidase reporter gene activity to test for the presence of recombinant virus plaques containing pBACgus3/BoNT/A-ED-PAR1Tb by incubating the washed monolayer with 2 mL of BacVector[®] Insect media containing 30 µL of 20 mg/mL X-Gluc Solution (EMD Biosciences-Novagen, Madison, WI) for approximately 2 hours in a 28 °C incubator.

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[0248] After identifying candidate recombinant virus plaques, several candidate virus plaques are eluted and plaque purified. To elute a recombinant virus, transfer a plug containing a recombinant virus plaque with a sterile Pasteur pipet to 1 mL BacVector[®] Insect media (EMD Biosciences-Novagen, Madison, WI) in a sterile screw-cap vial. Incubate the vial for approximately 2 hours at 22 °C or for approximately 16 hours at 4 °C. For each recombinant virus plaque, 2.5×10^5 Sf9 cells are plated in 35 mm culture dishes containing 2 mL of BacVector[®] Insect media (EMD Biosciences-Novagen, Madison, WI) and incubated for approximately 20 minutes in a 28 °C incubator. Remove the media and add 200 µL of eluted recombinant virus. After one hour, add 2 mL of 1% BacPlaque agarose-BacVector[®] Insect media containing 5% bovine serum albumin. After the agarose is solidified, add 1 mL BacVector[®] Insect media containing 5% bovine serum albumin to the transfected cells and transfer the cells to a 28 °C incubator for 3-5 days until plaques are visible. After 3-5 days post-transfection, plaques in the monolayer will be stained for β -glucuronidase reporter gene activity to test for the presence of recombinant virus plaques containing pBACgus3/BoNT/A-ED-PAR1Tb by incubating the washed monolayer with 2 mL of BacVector[®] Insect media containing 30 µL of 20 mg/mL X-Gluc Solution (EMD Biosciences-Novagen, Madison, WI) for approximately 2 hours in a 28 °C incubator.

[0249] To prepare a seed stock of virus, elute a recombinant virus by transferring a plug containing a recombinant virus plaque with a sterile Pasteur pipet to 1 mL BacVector[®] Insect media (EMD Biosciences-Novagen, Madison, WI) in a sterile screw-cap vial. Incubate the vial for approximately 16 hours at 4 °C. Approximately 5×10^5 Sf9 cells are plated in T-25 flask containing 5 mL of BacVector[®] Insect media (EMD Biosciences-Novagen, Madison, WI) and are incubated for approximately 20 minutes in a 28 °C incubator. Remove the media and add 300 µL of eluted recombinant virus. After one hour, add 5 mL BacVector[®] Insect media containing 5% bovine serum albumin to the transfected cells and transfer the cells to a 28 °C incubator for 3-5 days until the majority of cells become unattached and unhealthy. The virus is harvested by transferring the media to 15 mL snap-cap tubes and centrifuging tubes at 1000x g for 5 minutes to remove debris. The clarified supernatant is transferred to fresh 15 mL snap-cap tubes and are stored at 4 °C.

[0250] To prepare a high titer stock of virus, approximately 2×10^7 Sf9 cells are plated in T-75 flask containing 10 mL of BacVector[®] Insect media (EMD Biosciences-Novagen, Madison, WI) and are incubated for approximately 20 minutes in a 28 °C incubator. Remove the media and add 500 µL of virus seed stock. After one hour, add 10 mL BacVector[®] Insect media containing 5% bovine serum albumin to the transfected cells and transfer the cells to a 28 °C incubator for 3-5 days until the majority of cells become unattached and unhealthy. The virus is harvested by transferring the media to 15 mL snap-cap tubes and centrifuging tubes at 1000x g for 5 minutes to remove debris. The clarified supernatant is transferred to fresh 15 mL snap-cap tubes and are stored at 4 °C. High titer virus stocks should contain approximately 2×10^8 to 3×10^9 pfu of baculovirus.

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[0251] To express gp64-BoNT/A-ED-PAR1Tb using a baculoviral expression system, about 1.25×10^8 Sf9 cells are seeded in a 1 L flask containing 250 mL of BacVector[®] Insect media and are grown in an orbital shaker (150 rpm) to a cell density of approximately 5×10^8 . The culture is inoculated with approximately 2.5×10^9 of high titer stock recombinant baculovirus and incubated for approximately 48 hours in a 28 °C orbital shaker (150 rpm). Media is harvested by transferring the media to tubes and centrifuging tubes at 500x *g* for 5 minutes to remove debris. Media samples are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and expression is measured by Western blot analysis (as described in Example 5) using either anti-BoNT/A or anti-His antibodies in order to identify baculoviral stocks expressing BoNT/A-ED-PAR1Tb.

[0252] BoNT/A-ED-PAR1Tb is purified using the IMAC procedure, as described in Example 5. Expression from each culture is evaluated by a Bradford dye assay, polyacrylamide gel electrophoresis and Western blot analysis (as described in Example 5) in order to determine whether the amounts of BoNT/A-ED-PAR1Tb produced.

Example 8

Expression of Modified Clostridial Toxins in a Mammalian Cell

[0253] The following example illustrates a procedure useful for expressing any of the modified Clostridial toxins disclosed in the present specification in a mammalian cell.

[0254] To construct a suitable mammalian expression construct encoding a modified Clostridial toxin, restriction endonuclease sites suitable for cloning an operably linked polynucleotide molecule into a pSecTag2 vector (Invitrogen, Inc, Carlsbad, CA) are incorporated into the 5'- and 3' ends of the polynucleotide molecule SEQ ID NO: 136 encoding BoNT/A-ED-PAR1Tb of SEQ ID NO: 85. This polynucleotide molecule is synthesized and a pUCBHB1/BoNT/A-ED-PAR1Tb construct is obtained as described in Example 1. This construct is digested with restriction enzymes that 1) excise the insert containing the open reading frame of SEQ ID NO: 136 encoding BoNT/A-ED-PAR1Tb; and 2) enable this insert to be operably-linked to a pSecTag2 vector. This insert is subcloned using a T4 DNA ligase procedure into a pSecTag2 vector that is digested with appropriate restriction endonucleases to yield pSecTag2/ BoNT/A-ED-PAR1Tb. The ligation mixture is transformed into chemically competent *E. coli* DH5α cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 100 µg/mL of Ampicillin, and placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs are identified as Ampicillin resistant colonies. Candidate constructs are isolated using an alkaline lysis plasmid mini-preparation procedure and analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the insert. This cloning strategy yielded a pSecTag2 expression construct comprising the polynucleotide molecule of SEQ ID NO: 136 encoding the BoNT/A-ED-PAR1Tb of SEQ ID NO: 85 operably-linked to a carboxyl-terminal c-myc and polyhistidine binding peptides (FIG. 12).

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[0255] A similar cloning strategy is used to make pSecTag2 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 109 encoding BoNT/A-ED-PAR1Tb of SEQ ID NO: 85; SEQ ID NO: 110 or SEQ ID NO: 137 encoding BoNT/A-ED-PAR1Xa of SEQ ID NO: 86; the polynucleotide molecule of SEQ ID NO: 111 or SEQ ID NO: 138 encoding BoNT/A-ED-PAR2Tp of SEQ ID NO: 87; the polynucleotide molecule of SEQ ID NO: 112 or SEQ ID NO: 139 encoding BoNT/A-ED-PAR2Xa of SEQ ID NO: 88; the polynucleotide molecule of SEQ ID NO: 113 or SEQ ID NO: 140 encoding BoNT/A-ED-PAR3Tb of SEQ ID NO: 89; the polynucleotide molecule of SEQ ID NO: 114 or SEQ ID NO: 141 encoding BoNT/A-ED-PAR3Xa of SEQ ID NO: 90; the polynucleotide molecule of SEQ ID NO: 115 or SEQ ID NO: 142 encoding BoNT/A-ED-PAR4Tb of SEQ ID NO: 91; and the polynucleotide molecule of SEQ ID NO: 116 or SEQ ID NO: 143 encoding BoNT/A-ED-PAR4Xa of SEQ ID NO: 92.

[0256] A similar cloning strategy is used to make pSecTag2 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 117 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 93; SEQ ID NO: 118 or SEQ ID NO: 145 encoding BoNT/A-TD-PAR1Xa of SEQ ID NO: 94; the polynucleotide molecule of SEQ ID NO: 119 or SEQ ID NO: 146 encoding BoNT/A-TD-PAR2Tp of SEQ ID NO: 95; the polynucleotide molecule of SEQ ID NO: 120 or SEQ ID NO: 147 encoding BoNT/A-TD-PAR2Xa of SEQ ID NO: 96; the polynucleotide molecule of SEQ ID NO: 121 or SEQ ID NO: 148 encoding BoNT/A-TD-PAR1Tb of SEQ ID NO: 97; the polynucleotide molecule of SEQ ID NO: 122 or SEQ ID NO: 149 encoding BoNT/A-TD-PAR3Xa of SEQ ID NO: 98; the polynucleotide molecule of SEQ ID NO: 123 or SEQ ID NO: 150 encoding BoNT/A-TD-PAR4Tb of SEQ ID NO: 99; and the polynucleotide molecule of SEQ ID NO: 124 or SEQ ID NO: 151 encoding BoNT/A-TD-PAR4Xa of SEQ ID NO: 100.

[0257] A similar cloning strategy is used to make pSecTag2 expression constructs comprising the polynucleotide molecule of SEQ ID NO: 125 encoding BoNT/A-BD-PAR1Tb of SEQ ID NO: 101; the polynucleotide molecule of SEQ ID NO: 126 or SEQ ID NO: 153 encoding BoNT/A-BD-PAR1Xa of SEQ ID NO: 102; the polynucleotide molecule of SEQ ID NO: 127 or SEQ ID NO: 154 encoding BoNT/A-BD-PAR2Tp of SEQ ID NO: 103; the polynucleotide molecule of SEQ ID NO: 128 or SEQ ID NO: 155 encoding BoNT/A-BD-PAR2Xa of SEQ ID NO: 104; the polynucleotide molecule of SEQ ID NO: 129 or SEQ ID NO: 156 encoding BoNT/A-BD-PAR3Tb of SEQ ID NO: 105; the polynucleotide molecule of SEQ ID NO: 130 or SEQ ID NO: 157 encoding BoNT/A-BD-PAR3Xa of SEQ ID NO: 106; the polynucleotide molecule of SEQ ID NO: 131 or SEQ ID NO: 158 encoding BoNT/A-BD-PAR4Tb of SEQ ID NO: 107; and the polynucleotide molecule of SEQ ID NO: 132 or SEQ ID NO: 159 encoding BoNT/A-BD-PAR4Xa of SEQ ID NO: 108.

[0258] To transiently express modified Clostridial toxin in a cell line, about 1.5×10^5 SH-SY5Y cells are plated in a 35 mm tissue culture dish containing 3 mL of complete Dulbecco's Modified Eagle Media (DMEM), supplemented with 10% fetal bovine serum (FBS), 1x penicillin/streptomycin solution (Invitrogen, Inc, Carlsbad, CA) and 1x MEM non-essential amino acids solution (Invitrogen, Inc, Carlsbad,

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CA), and grown in a 37 °C incubator under 5% carbon dioxide until cells reach a density of about 5×10^5 cells/ml (6-16 hours). A 500 μ L transfection solution is prepared by adding 250 μ L of OPTI-MEM Reduced Serum Medium containing 15 μ L of LipofectAmine 2000 (Invitrogen, Carlsbad, CA) incubated at room temperature for 5 minutes to 250 μ L of OPTI-MEM Reduced Serum Medium containing 5 μ g of a pSecTag2 expression construct encoding a modified Clostridial toxin, such as, *e.g.*, pSecTag2/BoNT/A-ED-PAR1Tb. This transfection is incubated at room temperature for approximately 20 minutes. The complete, supplemented DMEM media is replaced with 2 mL of OPTI-MEM Reduced Serum Medium and the 500 μ L transfection solution is added to the SH-SY5Y cells and the cells are incubated in a 37 °C incubator under 5% carbon dioxide for approximately 6 to 18 hours. Transfection media is replaced with 3 mL of fresh complete, supplemented DMEM and the cells are incubated in a 37 °C incubator under 5% carbon dioxide for 48 hours. Both media and cells are collected for expression analysis of BoNT/A-ED-PAR1Tb. Media is harvested by transferring the media to 15 mL snap-cap tubes and centrifuging tubes at 500x *g* for 5 minutes to remove debris. Cells are harvested by rinsing cells once with 3.0 mL of 100 mM phosphate-buffered saline, pH 7.4 and lysing cells with a buffer containing 62.6 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloric acid (Tris-HCl), pH 6.8 and 2% sodium lauryl sulfate (SDS). Both media and cell samples are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and expression is measured by Western blot analysis (as described in Example 5) using either anti-BoNT/A, anti-c-myc or anti-His antibodies in order to identify pSecTag2 constructs expressing BoNT/A-ED-PAR1Tb. A similar procedure can be used to transiently express a pSecTag2 construct encoding any of the modified Clostridial toxin of SEQ ID NO: 86 to SEQ ID NO: 108.

[0259] To generate a stably-integrated cell line expressing a modified Clostridial toxin, approximately 1.5×10^5 SH-SY5Y cells are plated in a 35 mm tissue culture dish containing 3 mL of complete DMEM, supplemented with 10% FBS, 1x penicillin/streptomycin solution (Invitrogen, Inc, Carlsbad, CA) and 1x MEM non-essential amino acids solution (Invitrogen, Inc, Carlsbad, CA), and grown in a 37 °C incubator under 5% carbon dioxide until cells reach a density of about 5×10^5 cells/ml (6-16 hours). A 500 μ L transfection solution is prepared by adding 250 μ L of OPTI-MEM Reduced Serum Medium containing 15 μ L of LipofectAmine 2000 (Invitrogen, Carlsbad, CA) incubated at room temperature for 5 minutes to 250 μ L of OPTI-MEM Reduced Serum Medium containing 5 μ g of a pSecTag2 expression construct encoding a modified Clostridial toxin, such as, *e.g.*, pSecTag2/BoNT/A-ED-PAR1Tb. This transfection solution is incubated at room temperature for approximately 20 minutes. The complete, supplemented DMEM media is replaced with 2 mL of OPTI-MEM Reduced Serum Medium and the 500 μ L transfection solution is added to the SH-SY5Y cells and the cells are incubated in a 37 °C incubator under 5% carbon dioxide for approximately 6 to 18 hours. Transfection media is replaced with 3 mL of fresh complete, supplemented DMEM and cells are incubated in a 37 °C incubator under 5% carbon dioxide for approximately 48 hours. Media is replaced with 3 mL of fresh complete DMEM, containing approximately 5 μ g/mL of Zeocin™, 10% FBS, 1x penicillin/streptomycin solution (Invitrogen, Inc, Carlsbad, CA) and 1x MEM non-essential amino acids solution (Invitrogen, Inc, Carlsbad, CA). Cells are incubated in a 37 °C incubator under 5% carbon dioxide for approximately 3-4 weeks, with old media being replaced with fresh

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Zeocin™-selective, complete, supplemented DMEM every 4 to 5 days. Once Zeocin™-resistant colonies are established, resistant clones are replated to new 35 mm culture plates containing fresh complete DMEM, supplemented with approximately 5 µg/mL of Zeocin™, 10% FBS, 1x penicillin/streptomycin solution (Invitrogen, Inc, Carlsbad, CA) and 1x MEM non-essential amino acids solution (Invitrogen, Inc, Carlsbad, CA), until these cells reach a density of 6 to 20x10⁵ cells/mL. To test for expression of BoNT/A-ED-PAR1Tb from SH-SY5Y cell lines that have stably-integrated a pSecTag2/BoNT/A-ED-PAR1Tb, approximately 1.5x10⁵ SH-SY5Y cells from each cell line are plated in a 35 mm tissue culture dish containing 3 mL of Zeocin™-selective, complete, supplemented DMEM and grown in a 37 °C incubator under 5% carbon dioxide until cells reach a density of about 5x10⁵ cells/ml (6-16 hours). Media is replaced with 3 mL of fresh Zeocin™-selective, complete, supplemented DMEM and cells are incubated in a 37 °C incubator under 5% carbon dioxide for 48 hours. Both media and cells are collected for expression analysis of BoNT/A-c-myc-His. Media is harvested by transferring the media to 15 mL snap-cap tubes and centrifuging tubes at 500x *g* for 5 minutes to remove debris. Cells are harvest by rinsing cells once with 3.0 mL of 100 mM phosphate-buffered saline, pH 7.4 and lysing cells with a buffer containing 62.6 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloric acid (Tris-HCl), pH 6.8 and 2% sodium lauryl sulfate (SDS). Both media and cell samples are added to 2x LDS Sample Buffer (Invitrogen, Inc, Carlsbad, CA) and expression is measured by Western blot analysis (as described in Example 5) using either anti-BoNT/A, anti-c-myc or anti-His antibodies in order to identify SH-SY5Y cell lines expressing BoNT/A-ED-PAR1Tb. The established SH-SY5Y cell line showing the highest expression level of BoNT/A-ED-PAR1Tb is selected for large-scale expression using 3 L flasks. Procedures for large-scale expression are as outlined above except the starting volume is approximately 800-1000 mL of complete DMEM and concentrations of all reagents are proportionally increased for this volume. A similar procedure can be used to stably express a pSecTag2 construct encoding any of the modified Clostridial toxin of SEQ ID NO: 86 to SEQ ID NO: 108.

[0260] BoNT/A-ED-PAR1Tb is purified using the IMAC procedure, as described in Example 5. Expression from each culture is evaluated by a Bradford dye assay, polyacrylamide gel electrophoresis and Western blot analysis (as described in Example 5) in order to determine whether the amounts of BoNT/A-ED-PAR1Tb produced.

[0261] Although aspects of the present invention have been described with reference to the disclosed embodiments, one skilled in the art will readily appreciate that the specific examples disclosed are only illustrative of these aspects and in no way limit the present invention. Various modifications can be made without departing from the spirit of the present invention.

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What is claimed:

1. A modified Clostridial toxin comprising:
 - a) a PAR ligand domain;
 - b) a Clostridial toxin enzymatic domain;
 - c) a Clostridial toxin translocation domain; and
 - d) a Clostridial toxin binding domain.
2. The modified Clostridial toxin according to Claim 1, wherein the PAR ligand domain is operationally-linked to the amino terminus of the Clostridial toxin enzymatic domain.
3. The modified Clostridial toxin according to Claim 2, wherein the modified Clostridial toxin comprises an amino to carboxyl single polypeptide linear order comprising the PAR ligand domain, the Clostridial toxin enzymatic domain, the Clostridial toxin translocation domain and the Clostridial toxin binding domain.
4. The modified Clostridial toxin according to Claim 2, wherein the modified Clostridial toxin comprises an amino to carboxyl single polypeptide linear order comprising the PAR ligand domain, the Clostridial toxin enzymatic domain, the Clostridial toxin binding domain and the Clostridial toxin translocation domain.
5. The modified Clostridial toxin according to Claim 1, wherein the PAR ligand domain is operationally-linked to the amino terminus of the Clostridial toxin translocation domain.
6. The modified Clostridial toxin according to Claim 5, wherein the modified Clostridial toxin comprises an amino to carboxyl single polypeptide linear order comprising the Clostridial toxin binding domain, the Clostridial toxin enzymatic domain, the PAR ligand domain and the Clostridial toxin translocation domain.
7. The modified Clostridial toxin according to Claim 5, wherein the modified Clostridial toxin comprises an amino to carboxyl single polypeptide linear order comprising the Clostridial toxin enzymatic domain, the PAR ligand domain, the Clostridial toxin translocation domain and the Clostridial toxin binding domain.
8. The modified Clostridial toxin according to Claim 1, wherein the PAR ligand domain is operationally-linked to the amino terminus of the Clostridial toxin binding domain.
9. The modified Clostridial toxin according to Claim 8, wherein the modified Clostridial toxin comprises an amino to carboxyl single polypeptide linear order comprising the Clostridial toxin enzymatic domain, the PAR ligand domain, the Clostridial toxin binding domain and the Clostridial toxin translocation domain.
10. The modified Clostridial toxin according to Claim 1, wherein the modified Clostridial toxin further comprises a protease cleavage site; wherein cleavage of the protease cleavage site unmasks the PAR ligand domain.
11. The modified Clostridial toxin according to Claim 1, wherein the PAR ligand domain comprises a PAR1 ligand domain.
12. The modified Clostridial toxin according to Claim 11, wherein the PAR1 ligand domain comprises SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 or SEQ ID NO: 133.
13. The modified Clostridial toxin according to Claim 1, wherein the PAR ligand domain comprises a PAR2 ligand domain.

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14. The modified Clostridial toxin according to Claim 13, wherein the PAR2 ligand domain comprises SEQ ID NO: 24 or SEQ ID NO: 25.
15. The modified Clostridial toxin according to Claim 1, wherein the PAR ligand domain comprises a PAR3 ligand domain.
16. The modified Clostridial toxin according to Claim 15, wherein the PAR3 ligand domain comprises SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 134.
17. The modified Clostridial toxin according to Claim 1, wherein the PAR ligand domain comprises a PAR4 ligand domain.
18. The modified Clostridial toxin according to Claim 17, wherein the PAR4 ligand domain comprises SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 135 or SEQ ID NO: 160.
19. The modified Clostridial toxin according to Claim 1, wherein the modified Clostridial toxin is a modified Botulinum toxin comprising a PAR ligand domain, a Botulinum toxin enzymatic domain, a Botulinum toxin translocation domain and a Botulinum toxin binding domain.
20. The modified Clostridial toxin according to Claim 19, wherein the modified Botulinum toxin is a modified BoNT/A comprising a PAR ligand domain, a BoNT/A enzymatic domain, a BoNT/A translocation domain and a BoNT/A binding domain.
21. The modified Clostridial toxin according to Claim 19, wherein the modified Botulinum toxin is a modified BoNT/B comprising a PAR ligand domain, a BoNT/B enzymatic domain, a BoNT/B translocation domain and a BoNT/B binding domain.
22. The modified Clostridial toxin according to Claim 19, wherein the modified Botulinum toxin is a modified BoNT/C1 comprising a PAR ligand domain, a BoNT/C1 enzymatic domain, a BoNT/C1 translocation domain and a BoNT/C1 binding domain.
23. The modified Clostridial toxin according to Claim 19, wherein the modified Botulinum toxin is a modified BoNT/D comprising a PAR ligand domain, a BoNT/D enzymatic domain, a BoNT/D translocation domain and a BoNT/D binding domain.
24. The modified Clostridial toxin according to Claim 19, wherein the modified Botulinum toxin is a modified BoNT/E comprising a PAR ligand domain, a BoNT/E enzymatic domain, a BoNT/E translocation domain and a BoNT/E binding domain.
25. The modified Clostridial toxin according to Claim 19, wherein the modified Botulinum toxin is a modified BoNT/F comprising a PAR ligand domain, a BoNT/F enzymatic domain, a BoNT/F translocation domain and a BoNT/F binding domain.
26. The modified Clostridial toxin according to Claim 19, wherein the modified Botulinum toxin is a modified BoNT/G comprising a PAR ligand domain, a BoNT/G enzymatic domain, a BoNT/G translocation domain and a BoNT/G binding domain.
27. The modified Clostridial toxin according to Claim 1, wherein the modified Clostridial toxin is a modified Tetanus toxin comprising a PAR ligand domain, a Tetanus toxin enzymatic domain, a Tetanus toxin translocation domain and a Tetanus toxin binding domain.
28. A polynucleotide molecule encoding a modified Clostridial toxin, the polynucleotide molecule comprising:
 - a) polynucleotide molecule encoding a PAR ligand domain;
 - b) polynucleotide molecule encoding a Clostridial toxin enzymatic domain;

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- c) polynucleotide molecule encoding a Clostridial toxin translocation domain; and
 - d) polynucleotide molecule encoding a Clostridial toxin binding domain.
29. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encodes a polypeptide comprising the PAR ligand domain operationally-linked to the amino terminus of the Clostridial toxin enzymatic domain.
30. The polynucleotide molecule according to Claim 29, wherein the polynucleotide molecule encodes a modified Clostridial toxin comprising an amino to carboxyl single polypeptide linear order comprising the PAR ligand domain, the Clostridial toxin enzymatic domain, the Clostridial toxin translocation domain and the Clostridial toxin binding domain.
31. The polynucleotide molecule according to Claim 29, wherein the polynucleotide molecule encodes a modified Clostridial toxin comprising an amino to carboxyl single polypeptide linear order comprising the PAR ligand domain, the Clostridial toxin enzymatic domain, the Clostridial toxin binding domain and the Clostridial toxin translocation domain.
32. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encodes a polypeptide comprising the PAR ligand domain operationally-linked to the amino terminus of the Clostridial toxin translocation domain.
33. The polynucleotide molecule according to Claim 32, wherein the polynucleotide molecule encodes a modified Clostridial toxin comprising an amino to carboxyl single polypeptide linear order comprising the Clostridial toxin binding domain, the Clostridial toxin enzymatic domain, the PAR ligand domain and the Clostridial toxin translocation domain.
34. The polynucleotide molecule according to Claim 32, wherein the polynucleotide molecule encodes a modified Clostridial toxin comprising an amino to carboxyl single polypeptide linear order comprising the Clostridial toxin enzymatic domain, the PAR ligand domain, the Clostridial toxin translocation domain and the Clostridial toxin binding domain.
35. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encodes a polypeptide comprising the PAR ligand domain operationally-linked to the amino terminus of the Clostridial toxin binding domain.
36. The polynucleotide molecule according to Claim 35, wherein the polynucleotide molecule encodes a modified Clostridial toxin comprising an amino to carboxyl single polypeptide linear order comprising the Clostridial toxin enzymatic domain, the PAR ligand domain, the Clostridial toxin binding domain and the Clostridial toxin translocation domain.
37. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule further encodes a protease cleavage site; wherein cleavage of the protease cleavage site unmasks the PAR ligand domain.
38. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encoding the PAR ligand domain comprises a PAR1 ligand domain.
39. The polynucleotide molecule according to Claim 38, wherein the polynucleotide molecule encodes the PAR1 ligand domain comprising SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 or SEQ ID NO: 133.
40. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encoding the PAR ligand domain comprises a PAR2 ligand domain.
41. The polynucleotide molecule according to Claim 40, wherein the polynucleotide molecule encodes the PAR2 ligand domain comprises SEQ ID NO: 24 or SEQ ID NO: 25.

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42. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encoding the PAR ligand domain comprises a PAR3 ligand domain.
43. The polynucleotide molecule according to Claim 42, wherein the polynucleotide molecule encodes the PAR3 ligand domain comprises SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 134.
44. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encoding the PAR ligand domain comprises a PAR4 ligand domain.
45. The polynucleotide molecule according to Claim 44, wherein the polynucleotide molecule encodes the PAR4 ligand domain comprises SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 135 or SEQ ID NO: 160.
46. The polynucleotide molecule according to Claim 28, wherein the polynucleotide molecule encoding the modified Clostridial toxin comprises a polynucleotide molecule encoding a modified Botulinum toxin comprising a PAR ligand domain, a Botulinum toxin enzymatic domain, a Botulinum toxin translocation domain and a Botulinum toxin binding domain.
47. The modified Clostridial toxin according to Claim 46, wherein the polynucleotide molecule encoding the modified Botulinum toxin comprises a polynucleotide molecule encoding a modified BoNT/A comprising a PAR ligand domain, a BoNT/A enzymatic domain, a BoNT/A translocation domain and a BoNT/A binding domain.
48. The modified Clostridial toxin according to Claim 46, wherein the polynucleotide molecule encoding the modified Botulinum toxin comprises a polynucleotide molecule encoding a modified BoNT/B comprising a PAR ligand domain, a BoNT/B enzymatic domain, a BoNT/B translocation domain and a BoNT/B binding domain.
49. The modified Clostridial toxin according to Claim 46, wherein the polynucleotide molecule encoding the modified Botulinum toxin comprises a polynucleotide molecule encoding a modified BoNT/C1 comprising a PAR ligand domain, a BoNT/C1 enzymatic domain, a BoNT/C1 translocation domain and a BoNT/C1 binding domain.
50. The modified Clostridial toxin according to Claim 46, wherein the polynucleotide molecule encoding the modified Botulinum toxin comprises a polynucleotide molecule encoding a modified BoNT/D comprising a PAR ligand domain, a BoNT/D enzymatic domain, a BoNT/D translocation domain and a BoNT/D binding domain.
51. The modified Clostridial toxin according to Claim 46, wherein the polynucleotide molecule encoding the modified Botulinum toxin comprises a polynucleotide molecule encoding a modified BoNT/E comprising a PAR ligand domain, a BoNT/E enzymatic domain, a BoNT/E translocation domain and a BoNT/E binding domain.
52. The modified Clostridial toxin according to Claim 46, wherein the polynucleotide molecule encoding the modified Botulinum toxin comprises a polynucleotide molecule encoding a modified BoNT/F comprising a PAR ligand domain, a BoNT/F enzymatic domain, a BoNT/F translocation domain and a BoNT/F binding domain.
53. The modified Clostridial toxin according to Claim 46, wherein the polynucleotide molecule encoding the modified Botulinum toxin comprises a polynucleotide molecule encoding a modified BoNT/G comprising a PAR ligand domain, a BoNT/G enzymatic domain, a BoNT/G translocation domain and a BoNT/G binding domain.
54. The modified Clostridial toxin according to Claim 28, wherein the polynucleotide molecule encoding the modified Clostridial toxin comprises a polynucleotide molecule encoding a modified Tetanus toxin

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comprising a PAR ligand domain, a Tetanus toxin enzymatic domain, a Tetanus toxin translocation domain and a Tetanus toxin binding domain.

55. A method of producing a modified Clostridial toxin comprising the step of expressing a modified Clostridial toxin encoded by a polynucleotide molecule in a cell, wherein the modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain.
56. A methods of producing a modified Clostridial toxin comprising the steps of:
 - a. introducing into a cell a polynucleotide molecule encoding a modified Clostridial toxin comprising a PAR ligand domain; a Clostridial toxin enzymatic domain; a Clostridial toxin translocation domain; and a Clostridial toxin binding domain; and
 - b. expressing the modified Clostridial toxin encoded by the polynucleotide molecule.
57. A modified Clostridial toxin comprising:
 - a) a PAR ligand domain;
 - b) a Clostridial toxin enzymatic domain;
 - c) a Clostridial toxin translocation domain; and
 - d) a non-Clostridial toxin binding domain.
58. The modified Clostridial toxin according to Claim 57, wherein the non-Clostridial toxin binding domain is selected from the group consisting of a Nerve growth factor (NGF), a Leukemia inhibitory factor (LIF), a Basic fibroblast growth factor (bFGF), a Brain-derived neurotrophic factor (BDNF), a Neurotrophin-3 (NT-3), a Hydra head activator peptide (HHAP), a Transforming growth factor 1 (TGF-1), a Transforming growth factor 2 (TGF-2), a Transforming growth factor 3 (TGF-3), an Epidermal growth factor (EGF) or a Ciliary neurotrophic factor (CNTF).
59. The modified Clostridial toxin according to Claim 57, wherein the non-Clostridial toxin binding domain is selected from the group consisting of a Tumor necrosis factor (TNF-), an Interleukin-1 (IL-1), an Interleukin-1 (IL-1) or an Interleukin-8 (IL-8).
60. The modified Clostridial toxin according to Claim 57, wherein the non-Clostridial toxin binding domain is selected from the group consisting of a Bradykinin, a Dynorphin, a β -endorphin, an Etorphine, an Endomorphin-1, an Endomorphin-2, a Leu-enkephalin, a Met-enkephalin, a Galanin, a Lofentanil or a Nociceptin.
61. The modified Clostridial toxin according to Claim 57, wherein the non-Clostridial toxin binding domain is selected from the group consisting of an antibody against the lactoseries carbohydrate epitopes found on the surface of dorsal root ganglion neurons (e.g. monoclonal antibodies 1B2 and LA4), an antibody against any of the receptors for the binding domains given above or an antibody against the surface expressed antigen Thyl (e.g. monoclonal antibody MRC OX7).

FIG. 1.

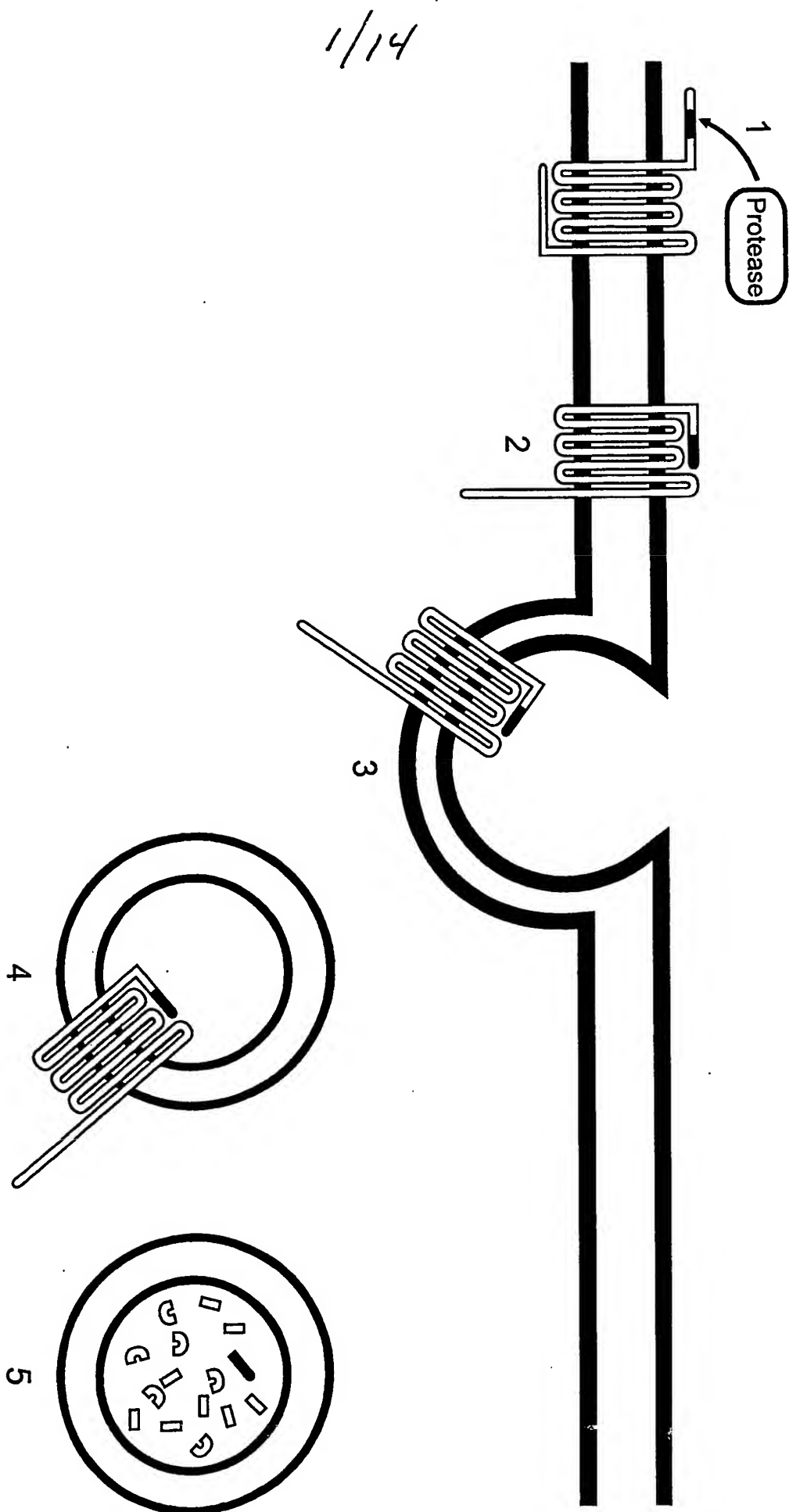
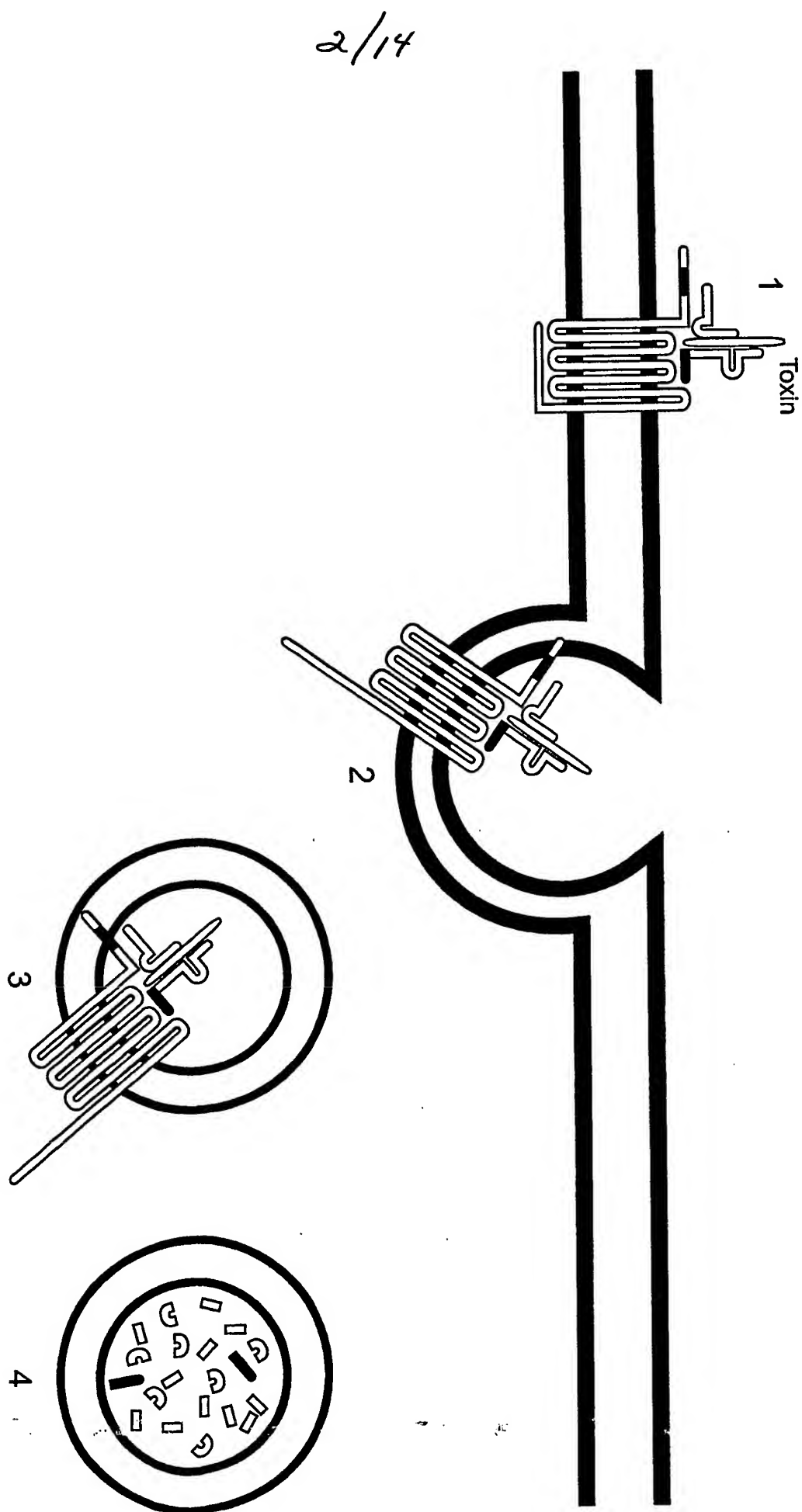
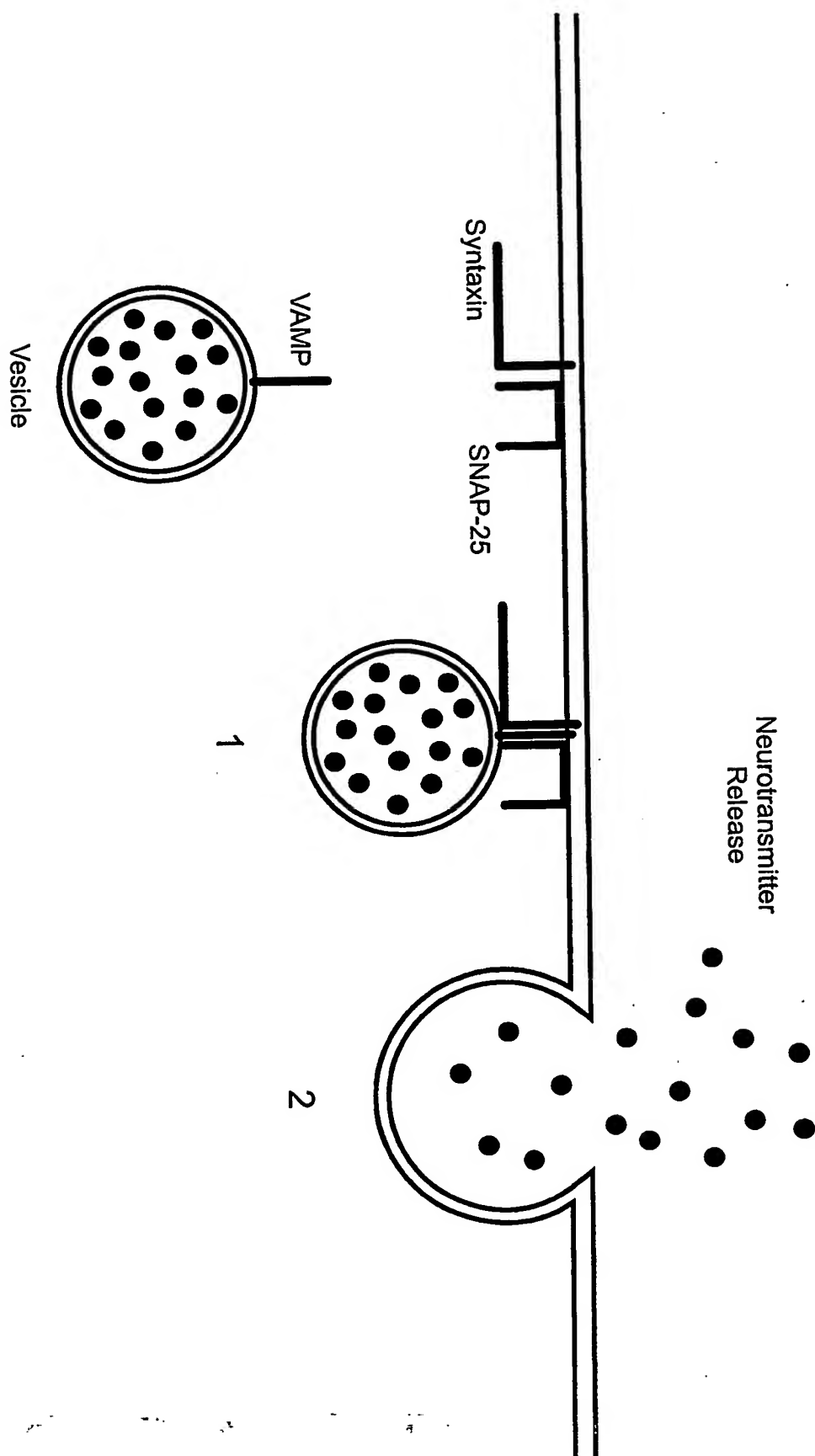


FIG. 2.



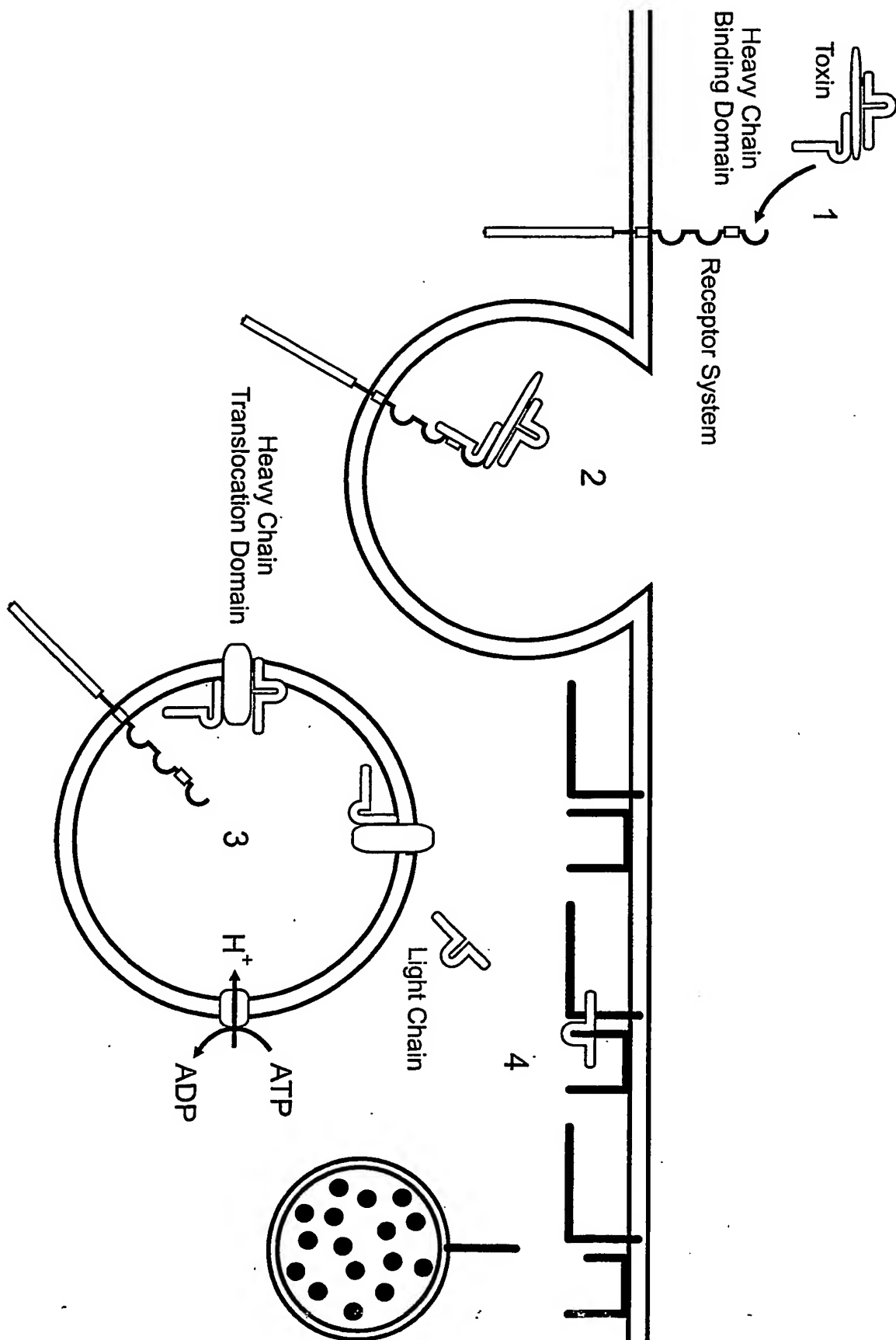
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FIG. 3a.



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FIG. 3b.



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FIG. 4A.

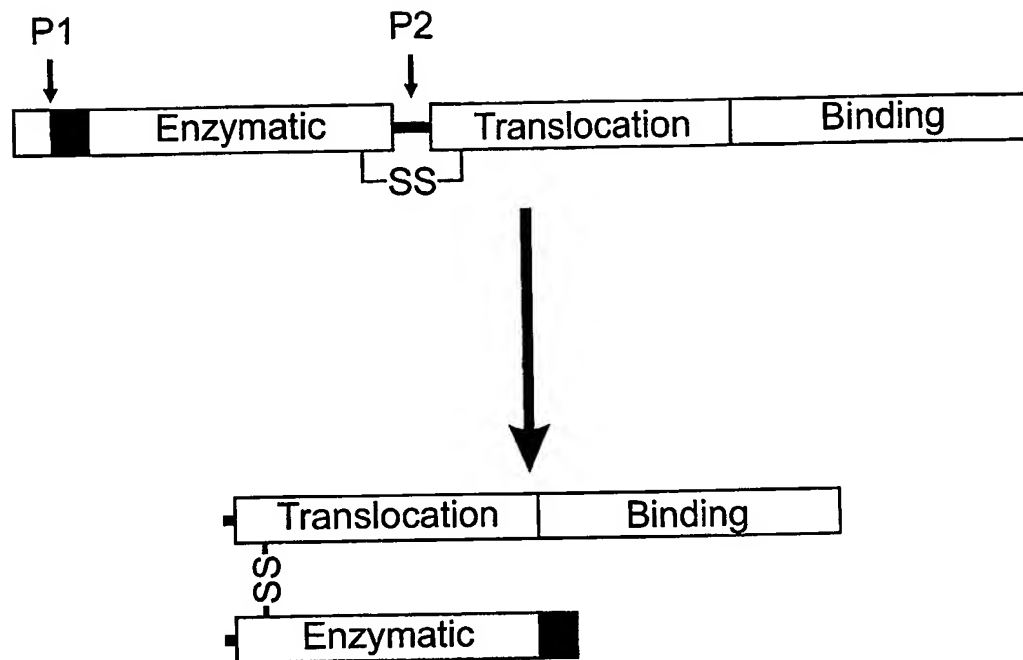
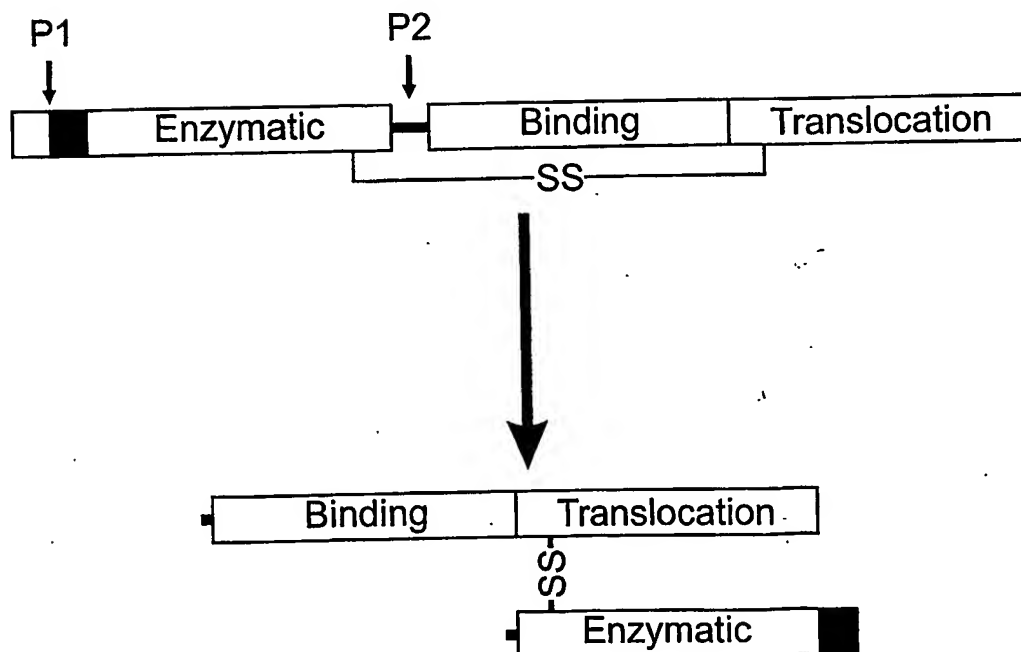


FIG. 4B.



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FIG. 4C.

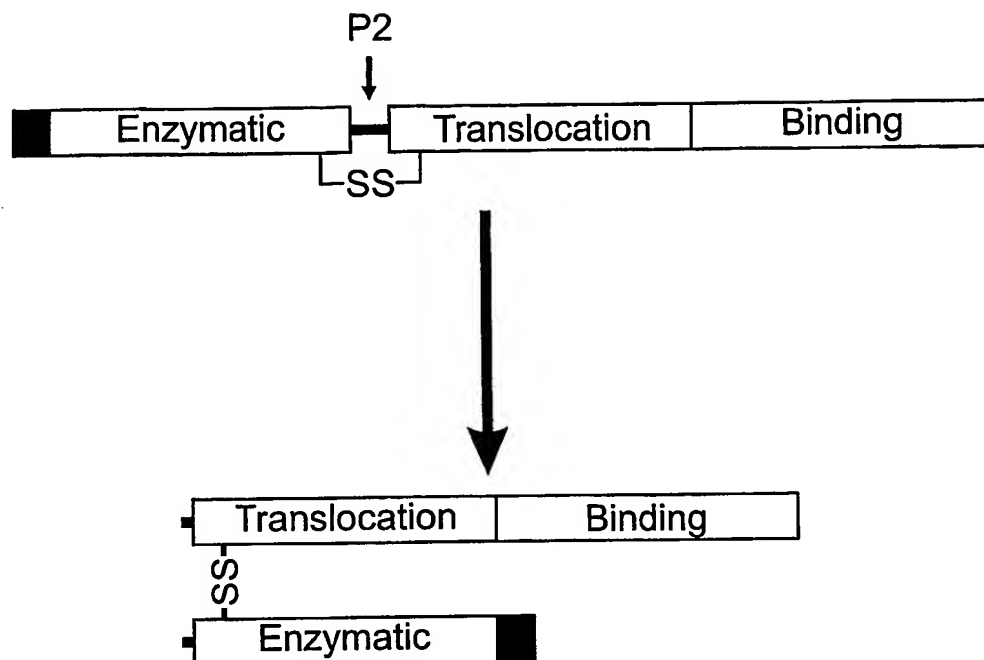
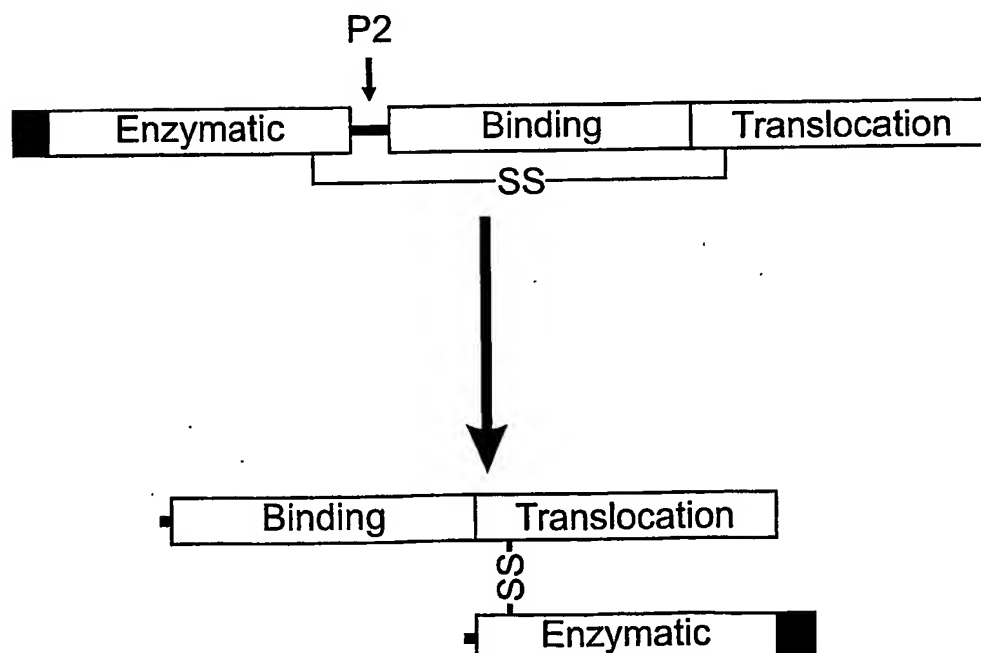


FIG. 4D.



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FIG. 5A.

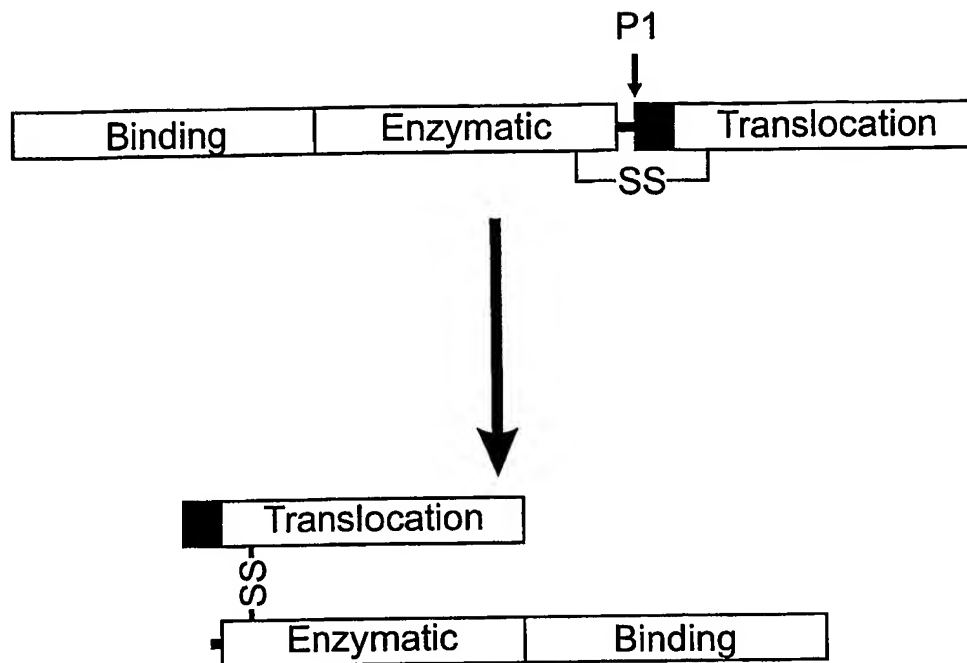
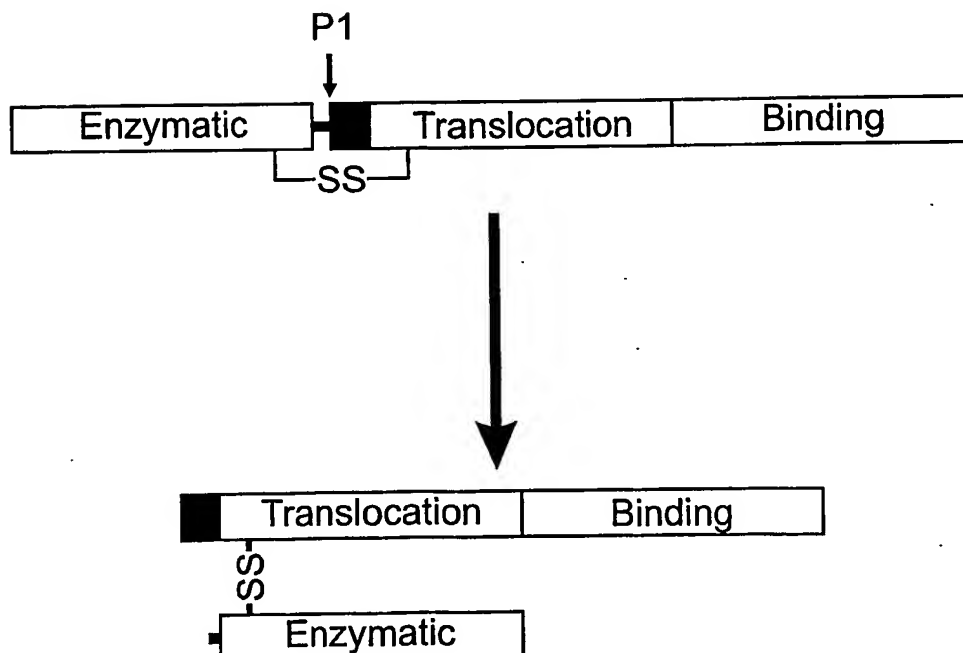
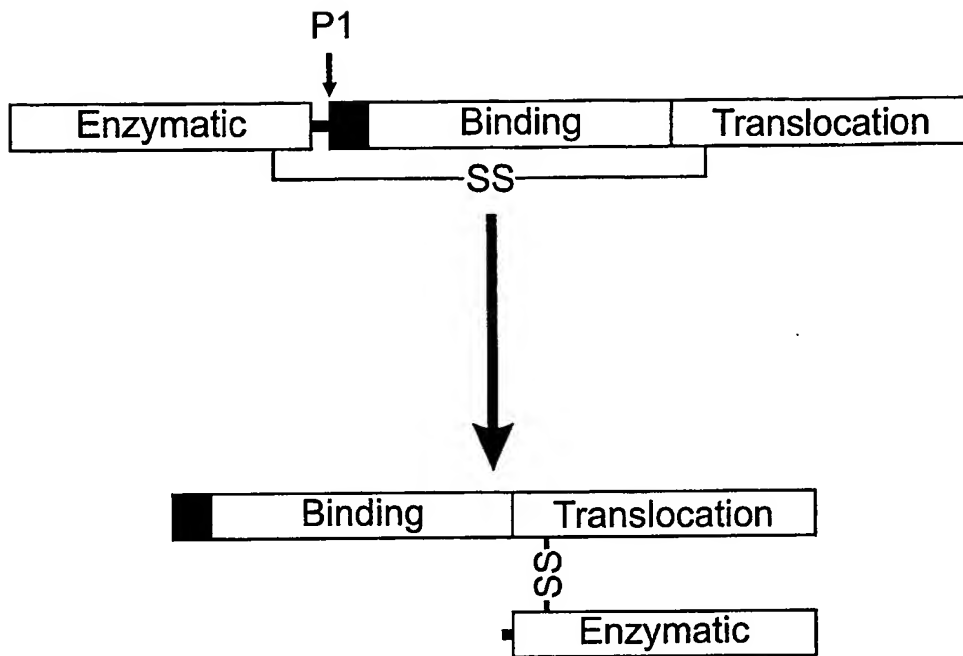


FIG. 5B.



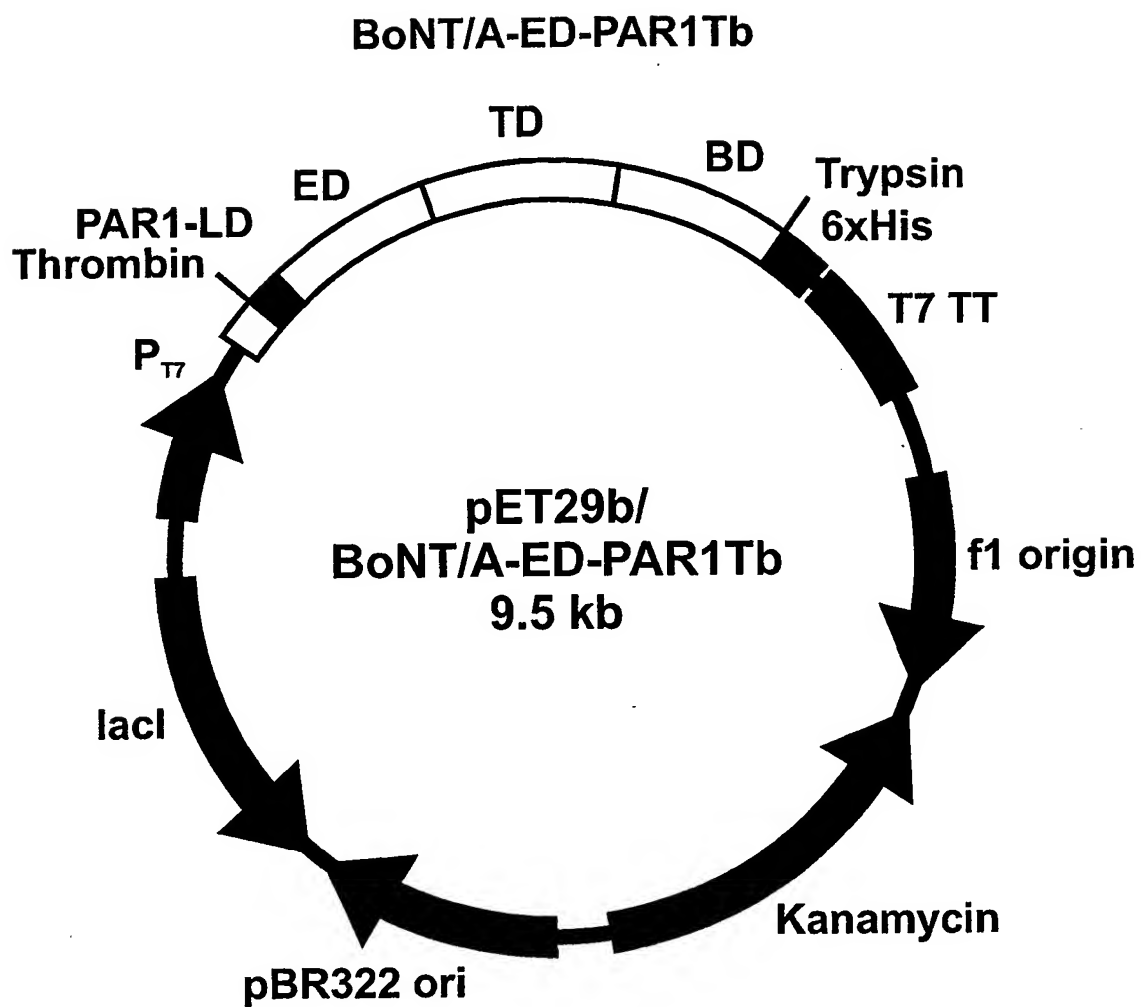
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FIG. 6.



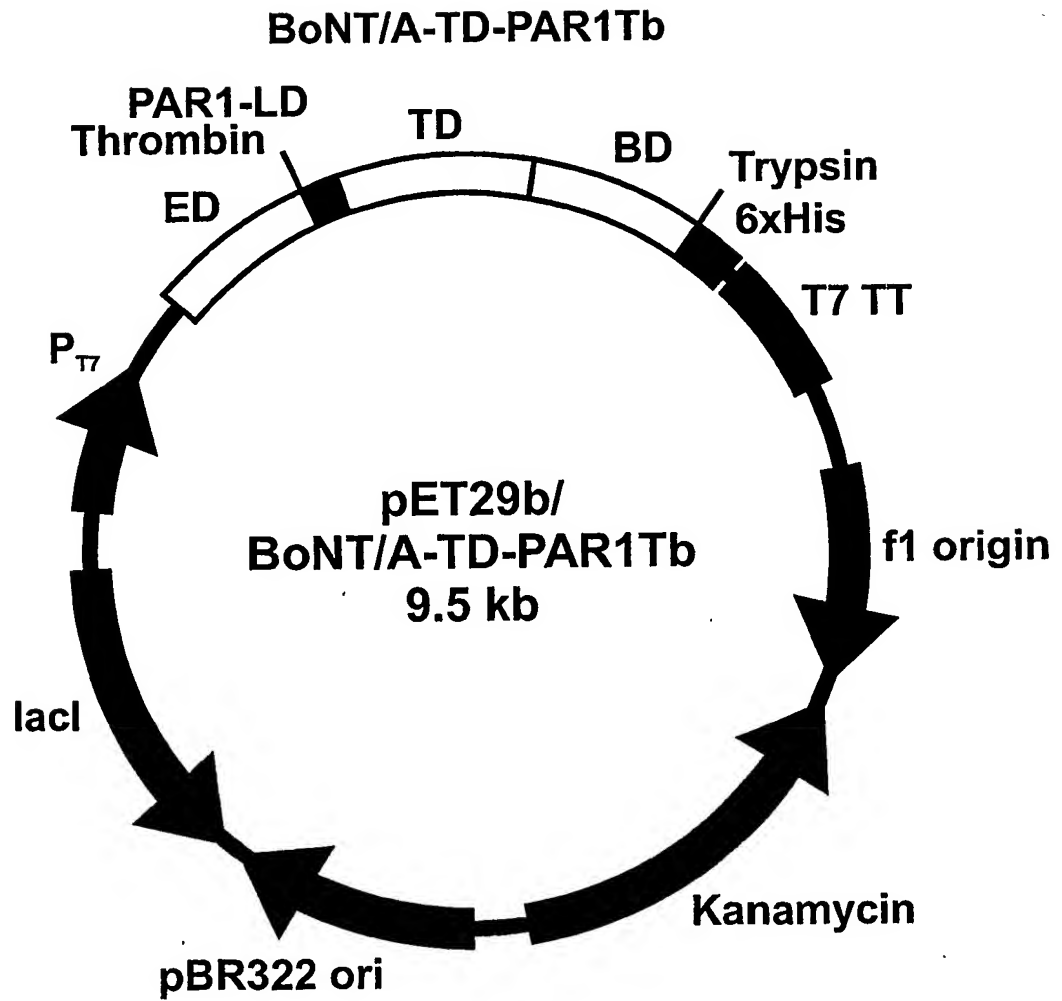
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FIG. 7.



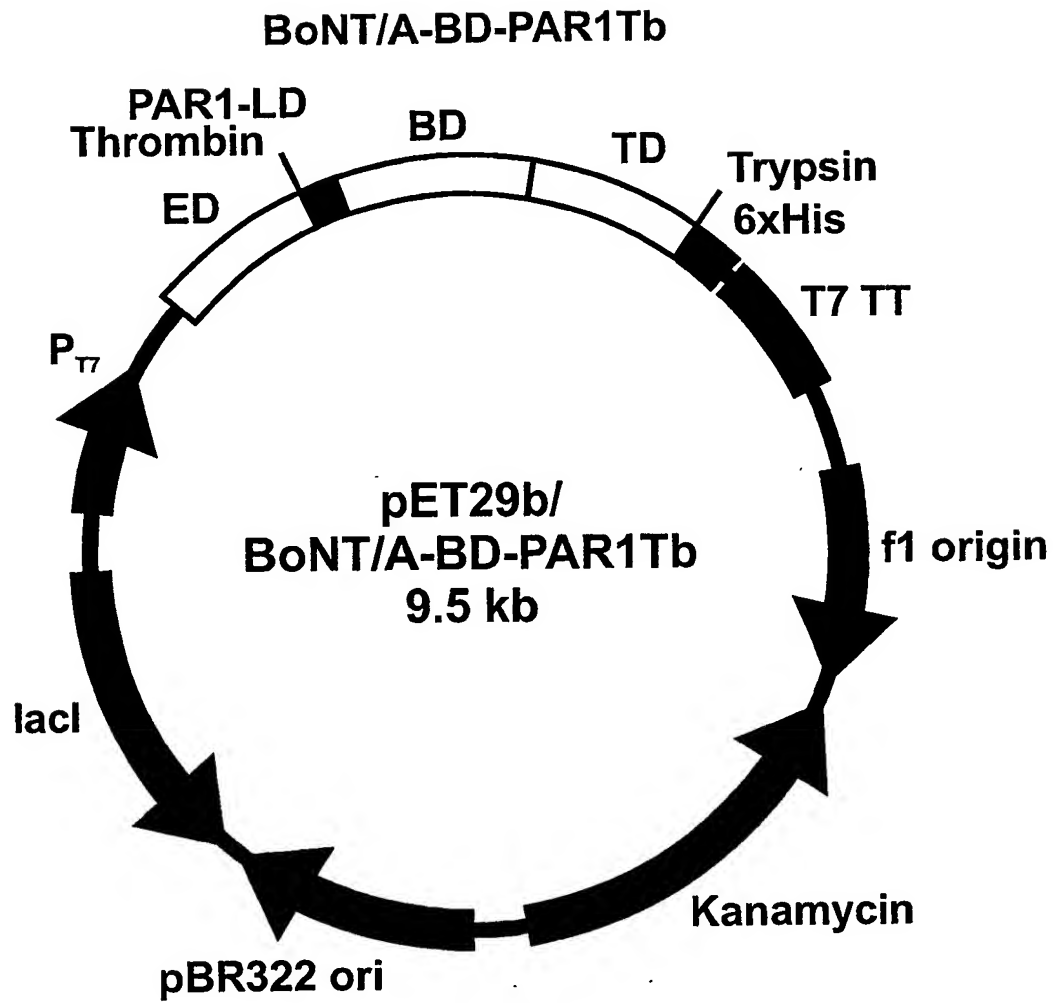
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FIG. 8.



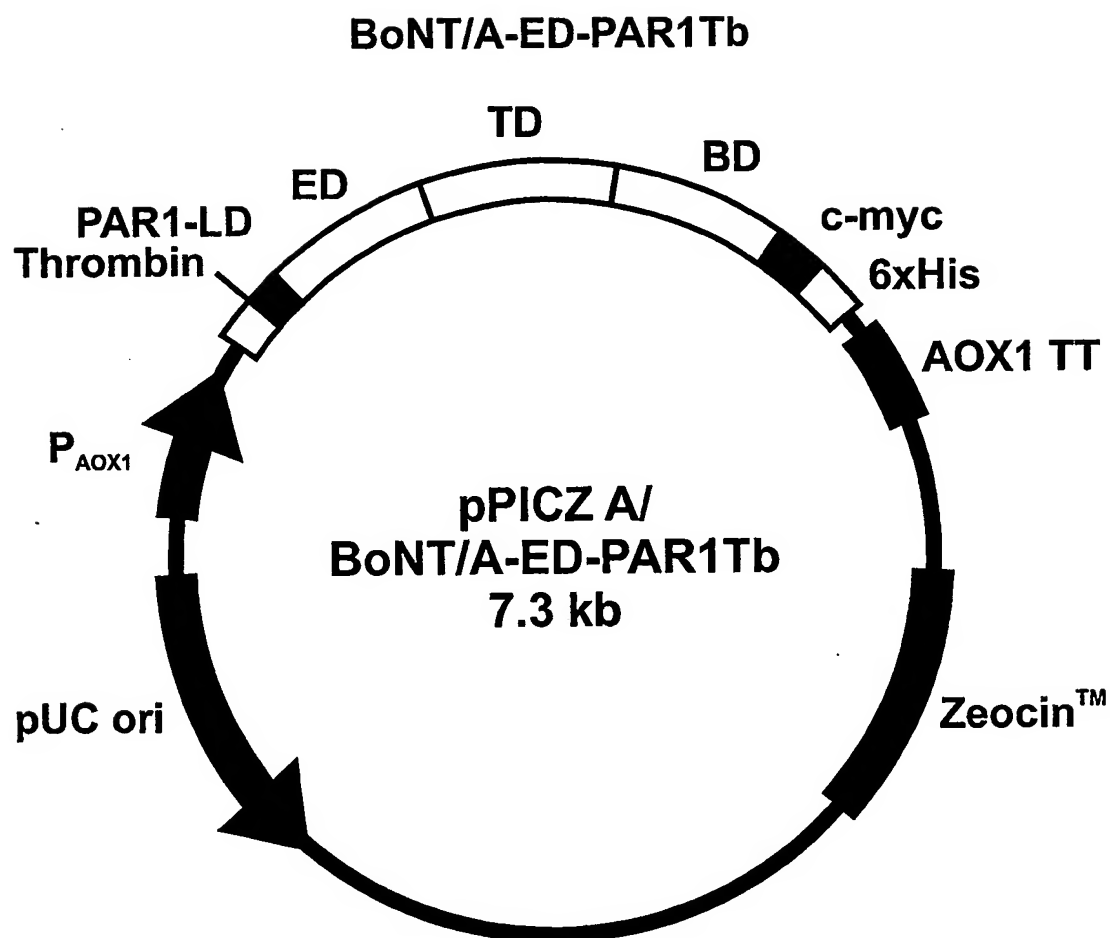
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FIG. 9.



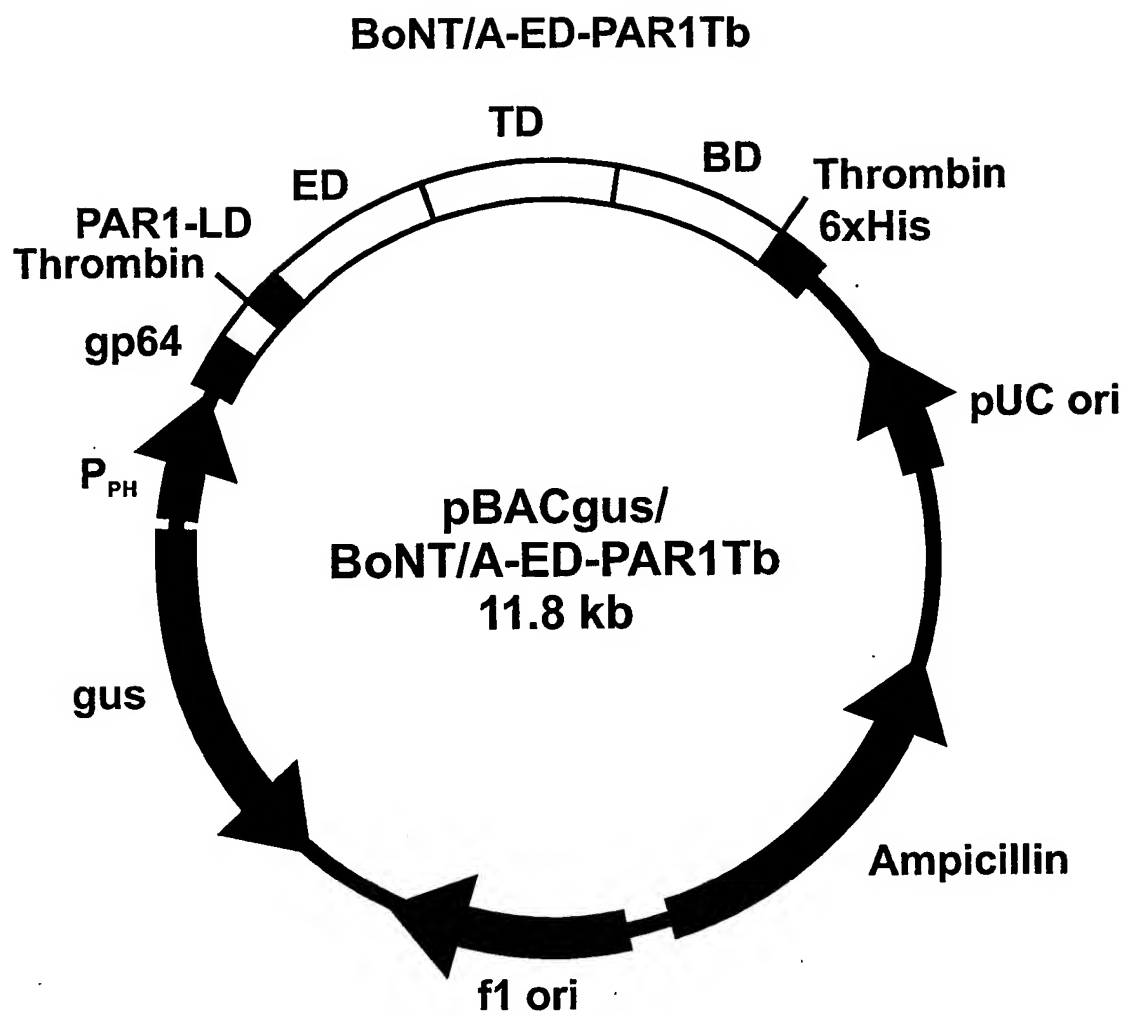
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FIG. 10.



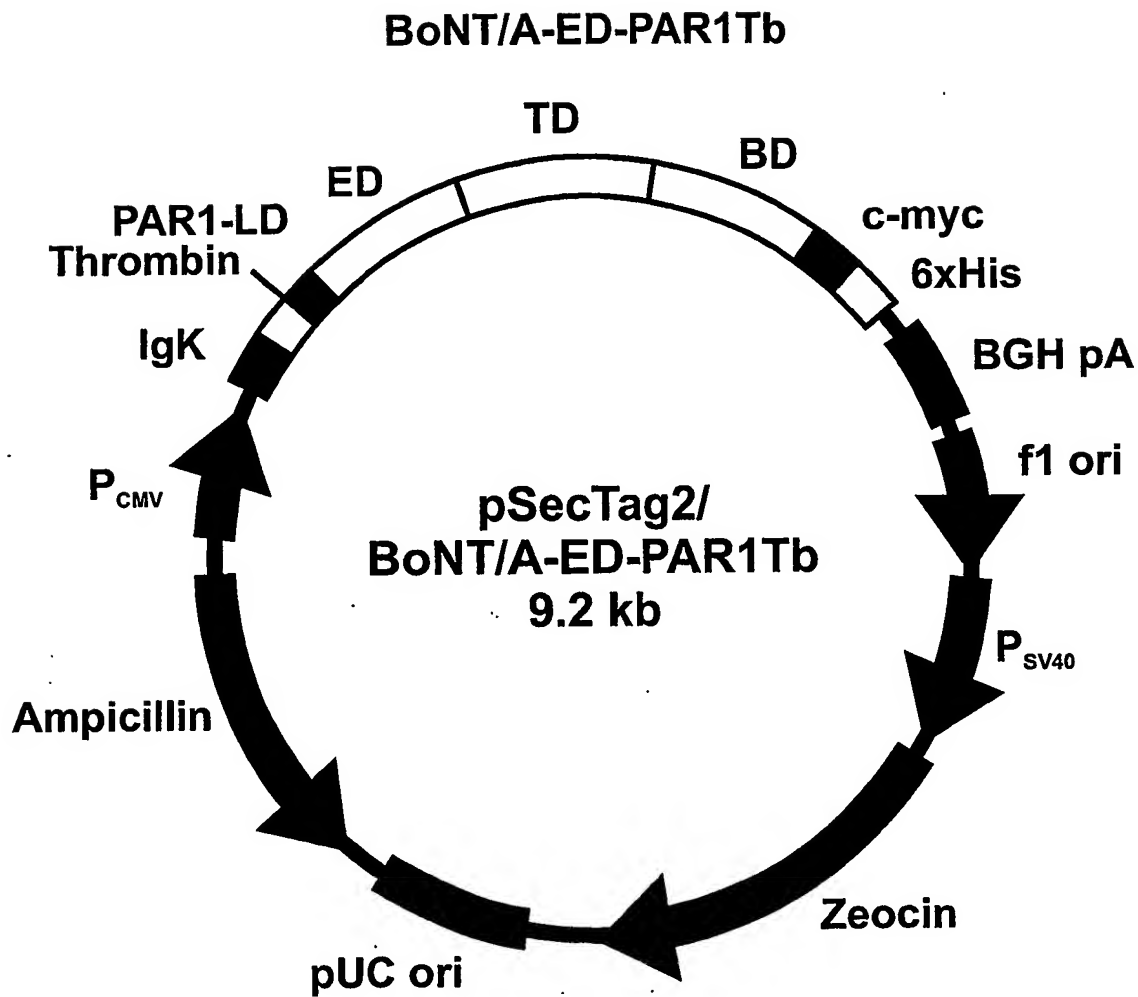
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FIG. 11.



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FIG. 12.



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SEQUENCE LISTING

<110> Li, Shengwen
 Steward, Lance E.
 Fernandez-Salas, Ester
 Gilmore, Marcella
 Francis, Joe
 Aoki, Kei Roger

<120> Degradable Clostridial Toxins

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<160> 160

<170> FastSEQ for Windows Version 4.0

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<221> DOMAIN
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 <223> Amino-terminal half of heavy chain comprising the translocation domain.

<221> DOMAIN
 <222> (861)...(1296)
 <223> Carboxyl-terminal half of heavy chain comprising the binding domain.

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 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
 35 40 45
 Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
 50 55 60
 Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
 65 70 75 80
 Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
 85 90 95
 Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
 100 105 110
 Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
 115 120 125
 Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
 130 135 140
 Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
 145 150 155 160
 Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr

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				165					170				175		
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
			180					185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
		195					200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215				220					
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225				230				235						240	
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
			245					250						255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
			260				265						270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
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Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
	290					295					300				
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305				310						315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
			325					330						335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
			340				345						350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
	355					360						365			
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370					375				380					
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385				390					395						400
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
			405					410						415	
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
			420					425					430		
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Asp	Lys	Gly	Tyr	Asn	Lys
		435					440					445			
Ala	Leu	Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe
	450					455					460				
Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu
465				470					475					480	
Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu
			485					490						495	
Asp	Leu	Ile	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	
			500				505					510			
Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu
	515						520					525			
Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu
	530					535					540				
Leu	Asp	Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu
545				550					555						560
His	Gly	Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu
			565					570						575	
Leu	Asn	Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys
		580					585						590		
Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu
	595					600						605			
Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr
	610					615					620				
Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala

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625		630		635		640
Leu Asn Ile Gly	Asn Met Leu Tyr Lys Asp	Asp Phe Val Gly	Ala Leu			
	645		650		655	
Ile Phe Ser Gly	Ala Val Ile Leu Leu Glu	Phe Ile Pro Glu	Ile Ala			
	660		665		670	
Ile Pro Val Leu	Gly Thr Phe Ala Leu Val Ser Tyr	Ile Ala Asn Lys				
	675		680		685	
Val Leu Thr Val	Gln Thr Ile Asp Asn Ala Leu Ser Lys	Arg Asn Glu				
	690		695		700	
Lys Trp Asp Glu	Val Tyr Lys Tyr Ile Val Thr Asn Trp	Leu Ala Lys				
705		710		715		720
Val Asn Thr Gln	Ile Asp Leu Ile Arg Lys Lys Met Lys	Glu Ala Leu				
	725		730		735	
Glu Asn Gln Ala	Glu Ala Thr Lys Ala Ile Ile Asn Tyr	Gln Tyr Asn				
	740		745		750	
Gln Tyr Thr Glu	Glu Glu Lys Asn Asn Ile Asn Phe Asn	Ile Asp Asp				
	755		760		765	
Leu Ser Ser Lys	Leu Asn Glu Ser Ile Asn Lys Ala Met	Ile Asn Ile				
	770		775		780	
Asn Lys Phe Leu	Asn Gln Cys Ser Val Ser Tyr Leu Met	Asn Ser Met				
785		790		795		800
Ile Pro Tyr Gly	Val Lys Arg Leu Glu Asp Phe Asp	Ala Ser Leu Lys				
	805		810		815	
Asp Ala Leu Leu	Lys Tyr Ile Tyr Asp Asn Arg Gly Thr	Leu Ile Gly				
	820		825		830	
Gln Val Asp Arg	Leu Lys Asp Lys Val Asn Asn Thr Leu Ser	Thr Asp				
	835		840		845	
Ile Pro Phe Gln	Leu Ser Lys Tyr Val Asp Asn Gln Arg	Leu Leu Ser				
	850		855		860	
Thr Phe Thr Glu	Tyr Ile Lys Asn Ile Ile Asn Thr Ser	Ile Leu Asn				
865		870		875		880
Leu Arg Tyr Glu	Ser Asn His Leu Ile Asp Leu Ser Arg Tyr	Ala Ser				
	885		890		895	
Lys Ile Asn Ile	Gly Ser Lys Val Asn Phe Asp Pro Ile Asp	Lys Asn				
	900		905		910	
Gln Ile Gln Leu	Phe Asn Leu Glu Ser Ser Lys Ile Glu	Val Ile Leu				
	915		920		925	
Lys Asn Ala Ile	Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser	Thr Ser				
	930		935		940	
Phe Trp Ile Arg	Ile Pro Lys Tyr Phe Asn Ser Ile Ser	Leu Asn Asn				
945		950		955		960
Glu Tyr Thr Ile	Ile Asn Cys Met Glu Asn Asn Ser Gly	Trp Lys Val				
	965		970		975	
Ser Leu Asn Tyr	Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr	Gln Glu				
	980		985		990	
Ile Lys Gln Arg	Val Val Phe Lys Tyr Ser Gln Met Ile Asn	Ile Ser				
	995		1000		1005	
Asp Tyr Ile Asn	Arg Trp Ile Phe Val Thr Ile Thr Asn Asn	Arg Leu				
	1010		1015		1020	
Asn Asn Ser Lys	Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln	Lys Pro				
1025		1030		1035		1040
Ile Ser Asn Leu	Gly Asn Ile His Ala Ser Asn Asn Ile Met	Phe Lys				
	1045		1050		1055	
Leu Asp Gly Cys	Arg Asp Thr His Arg Tyr Ile Trp Ile Lys	Tyr Phe				
	1060		1065		1070	
Asn Leu Phe Asp	Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp	Leu Tyr				
	1075		1080		1085	
Asp Asn Gln Ser	Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly	Asp Tyr				

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1090	1095	1100
Leu Gln Tyr Asp Lys	Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn	
1105	1110	1115
Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu		1120
	1125	1130
Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser		1135
	1140	1145
Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly		1150
	1155	1160
Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val		1165
	1170	1175
Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala		1180
1185	1190	1195
Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn		1200
	1205	1210
Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr		1215
	1220	1225
Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly		1230
	1235	1240
Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser		1245
	1250	1255
Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys		1260
1265	1270	1275
Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu Arg Pro Leu		1280
	1285	1290
		1295

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<223> Carboxyl-terminal half of heavy chain comprising the binding domain.

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Asn Asn Ile Ile Met Met Glu Pro Pro Phe Ala Arg Gly Thr Gly Arg	
20 25 30	
Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Ile Pro Glu	
35 40 45	
Arg Tyr Thr Phe Gly Tyr Lys Pro Glu Asp Phe Asn Lys Ser Ser Gly	
50 55 60	
Ile Phe Asn Arg Asp Val Cys Glu Tyr Tyr Asp Pro Asp Tyr Leu Asn	
65 70 75 80	
Thr Asn Asp Lys Lys Asn Ile Phe Leu Gln Thr Met Ile Lys Leu Phe	

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				85				90				95			
Asn	Arg	Ile	Lys	Ser	Lys	Pro	Leu	Gly	Glu	Lys	Leu	Leu	Glu	Met	Ile
			100					105					110		
Ile	Asn	Gly	Ile	Pro	Tyr	Leu	Gly	Asp	Arg	Arg	Val	Pro	Leu	Glu	Glu
		115					120					125			
Phe	Asn	Thr	Asn	Ile	Ala	Ser	Val	Thr	Val	Asn	Lys	Leu	Ile	Ser	Asn
		130					135				140				
Pro	Gly	Glu	Val	Glu	Arg	Lys	Lys	Gly	Ile	Phe	Ala	Asn	Leu	Ile	Ile
145					150					155					160
Phe	Gly	Pro	Gly	Pro	Val	Leu	Asn	Glu	Asn	Glu	Thr	Ile	Asp	Ile	Gly
				165						170					175
Ile	Gln	Asn	His	Phe	Ala	Ser	Arg	Glu	Gly	Phe	Gly	Gly	Ile	Met	Gln
			180					185					190		
Met	Lys	Phe	Cys	Pro	Glu	Tyr	Val	Ser	Val	Phe	Asn	Asn	Val	Gln	Glu
		195					200					205			
Asn	Lys	Gly	Ala	Ser	Ile	Phe	Asn	Arg	Arg	Gly	Tyr	Phe	Ser	Asp	Pro
		210					215				220				
Ala	Leu	Ile	Leu	Met	His	Glu	Leu	Ile	His	Val	Leu	His	Gly	Leu	Tyr
225					230					235					240
Gly	Ile	Lys	Val	Asp	Asp	Leu	Pro	Ile	Val	Pro	Asn	Glu	Lys	Lys	Phe
				245						250					255
Phe	Met	Gln	Ser	Thr	Asp	Ala	Ile	Gln	Ala	Glu	Glu	Leu	Tyr	Thr	Phe
			260					265					270		
Gly	Gly	Gln	Asp	Pro	Ser	Ile	Ile	Thr	Pro	Ser	Thr	Asp	Lys	Ser	Ile
		275					280					285			
Tyr	Asp	Lys	Val	Leu	Gln	Asn	Phe	Arg	Gly	Ile	Val	Asp	Arg	Leu	Asn
	290					295					300				
Lys	Val	Leu	Val	Cys	Ile	Ser	Asp	Pro	Asn	Ile	Asn	Ile	Asn	Ile	Tyr
305					310					315					320
Lys	Asn	Lys	Phe	Lys	Asp	Lys	Tyr	Lys	Phe	Val	Glu	Asp	Ser	Glu	Gly
			325							330					335
Lys	Tyr	Ser	Ile	Asp	Val	Glu	Ser	Phe	Asp	Lys	Leu	Tyr	Lys	Ser	Leu
			340					345					350		
Met	Phe	Gly	Phe	Thr	Glu	Thr	Asn	Ile	Ala	Glu	Asn	Tyr	Lys	Ile	Lys
		355					360					365			
Thr	Arg	Ala	Ser	Tyr	Phe	Ser	Asp	Ser	Leu	Pro	Pro	Val	Lys	Ile	Lys
	370					375					380				
Asn	Leu	Leu	Asp	Asn	Glu	Ile	Tyr	Thr	Ile	Glu	Glu	Gly	Phe	Asn	Ile
385					390					395					400
Ser	Asp	Lys	Asp	Met	Glu	Lys	Glu	Tyr	Arg	Gly	Gln	Asn	Lys	Ala	Ile
			405						410						415
Asn	Lys	Gln	Ala	Tyr	Glu	Glu	Ile	Ser	Lys	Glu	His	Leu	Ala	Val	Tyr
			420					425					430		
Lys	Ile	Gln	Met	Cys	Lys	Ser	Val	Lys	Ala	Pro	Gly	Ile	Cys	Ile	Asp
		435					440					445			
Val	Asp	Asn	Glu	Asp	Leu	Phe	Phe	Ile	Ala	Asp	Lys	Asn	Ser	Phe	Ser
	450					455					460				
Asp	Asp	Leu	Ser	Lys	Asn	Glu	Arg	Ile	Glu	Tyr	Asn	Thr	Gln	Ser	Asn
465					470					475					480
Tyr	Ile	Glu	Asn	Asp	Phe	Pro	Ile	Asn	Glu	Leu	Ile	Leu	Asp	Thr	Asp
			485						490						495
Leu	Ile	Ser	Lys	Ile	Glu	Leu	Pro	Ser	Glu	Asn	Thr	Glu	Ser	Leu	Thr
			500					505					510		
Asp	Phe	Asn	Val	Asp	Val	Pro	Val	Tyr	Glu	Lys	Gln	Pro	Ala	Ile	Lys
		515					520					525			
Lys	Ile	Phe	Thr	Asp	Glu	Asn	Thr	Ile	Phe	Gln	Tyr	Leu	Tyr	Ser	Gln
	530					535					540				
Thr	Phe	Pro	Leu	Asp	Ile	Arg	Asp	Ile	Ser	Leu	Thr	Ser	Ser	Phe	Asp
545					550					555					560

Li et al., Degradable Clostridial Toxins

Asp	Ala	Leu	Leu	Phe	Ser	Asn	Lys	Val	Tyr	Ser	Phe	Phe	Ser	Met	Asp
				565					570					575	
Tyr	Ile	Lys	Thr	Ala	Asn	Lys	Val	Val	Glu	Ala	Gly	Leu	Phe	Ala	Gly
			580					585					590		
Trp	Val	Lys	Gln	Ile	Val	Asn	Asp	Phe	Val	Ile	Glu	Ala	Asn	Lys	Ser
		595					600					605			
Asn	Thr	Met	Asp	Lys	Ile	Ala	Asp	Ile	Ser	Leu	Ile	Val	Pro	Tyr	Ile
	610					615					620				
Gly	Leu	Ala	Leu	Asn	Val	Gly	Asn	Glu	Thr	Ala	Lys	Gly	Asn	Phe	Glu
625				630						635					640
Asn	Ala	Phe	Glu	Ile	Ala	Gly	Ala	Ser	Ile	Leu	Leu	Glu	Phe	Ile	Pro
			645					650						655	
Glu	Leu	Leu	Ile	Pro	Val	Val	Gly	Ala	Phe	Leu	Leu	Glu	Ser	Tyr	Ile
		660					665						670		
Asp	Asn	Lys	Asn	Lys	Ile	Ile	Lys	Thr	Ile	Asp	Asn	Ala	Leu	Thr	Lys
		675					680					685			
Arg	Asn	Glu	Lys	Trp	Ser	Asp	Met	Tyr	Gly	Leu	Ile	Val	Ala	Gln	Trp
	690					695					700				
Leu	Ser	Thr	Val	Asn	Thr	Gln	Phe	Tyr	Thr	Ile	Lys	Glu	Gly	Met	Tyr
705				710						715					720
Lys	Ala	Leu	Asn	Tyr	Gln	Ala	Gln	Ala	Leu	Glu	Glu	Ile	Ile	Lys	Tyr
			725					730						735	
Arg	Tyr	Asn	Ile	Tyr	Ser	Glu	Lys	Glu	Lys	Ser	Asn	Ile	Asn	Ile	Asp
		740					745					750			
Phe	Asn	Asp	Ile	Asn	Ser	Lys	Leu	Asn	Glu	Gly	Ile	Asn	Gln	Ala	Ile
	755					760					765				
Asp	Asn	Ile	Asn	Asn	Phe	Ile	Asn	Gly	Cys	Ser	Val	Ser	Tyr	Leu	Met
	770				775					780					
Lys	Lys	Met	Ile	Pro	Leu	Ala	Val	Glu	Lys	Leu	Leu	Asp	Phe	Asp	Asn
785				790						795					800
Thr	Leu	Lys	Lys	Asn	Leu	Leu	Asn	Tyr	Ile	Asp	Glu	Asn	Lys	Leu	Tyr
			805						810					815	
Leu	Ile	Gly	Ser	Ala	Glu	Tyr	Glu	Lys	Ser	Lys	Val	Asn	Lys	Tyr	Leu
		820					825					830			
Lys	Thr	Ile	Met	Pro	Phe	Asp	Leu	Ser	Ile	Tyr	Thr	Asn	Asp	Thr	Ile
	835						840					845			
Leu	Ile	Glu	Met	Phe	Asn	Lys	Tyr	Asn	Ser	Glu	Ile	Leu	Asn	Asn	Ile
	850					855					860				
Ile	Leu	Asn	Leu	Arg	Tyr	Lys	Asp	Asn	Asn	Leu	Ile	Asp	Leu	Ser	Gly
865				870						875					880
Tyr	Gly	Ala	Lys	Val	Glu	Val	Tyr	Asp	Gly	Val	Glu	Leu	Asn	Asp	Lys
			885					890						895	
Asn	Gln	Phe	Lys	Leu	Thr	Ser	Ser	Ala	Asn	Ser	Lys	Ile	Arg	Val	Thr
		900						905					910		
Gln	Asn	Gln	Asn	Ile	Ile	Phe	Asn	Ser	Val	Phe	Leu	Asp	Phe	Ser	Val
		915					920					925			
Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr	Lys	Asn	Asp	Gly	Ile	Gln	Asn
	930					935					940				
Tyr	Ile	His	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met	Lys	Asn	Asn	Ser
945				950						955					960
Gly	Trp	Lys	Ile	Ser	Ile	Arg	Gly	Asn	Arg	Ile	Ile	Trp	Thr	Leu	Ile
			965					970						975	
Asp	Ile	Asn	Gly	Lys	Thr	Lys	Ser	Val	Phe	Phe	Glu	Tyr	Asn	Ile	Arg
		980					985						990		
Glu	Asp	Ile	Ser	Glu	Tyr	Ile	Asn	Arg	Trp	Phe	Phe	Val	Thr	Ile	Thr
	995						1000					1005			
Asn	Asn	Leu	Asn	Asn	Ala	Lys	Ile	Tyr	Ile	Asn	Gly	Lys	Leu	Glu	Ser
	1010					1015					1020				
Asn	Thr	Asp	Ile	Lys	Asp	Ile	Arg	Glu	Val	Ile	Ala	Asn	Gly	Glu	Ile

Li et al., Degradable Clostridial Toxins

1025					1030					1035					1040
Ile	Phe	Lys	Leu	Asp	Gly	Asp	Ile	Asp	Arg	Thr	Gln	Phe	Ile	Trp	Met
				1045					1050					1055	
Lys	Tyr	Phe	Ser	Ile	Phe	Asn	Thr	Glu	Leu	Ser	Gln	Ser	Asn	Ile	Glu
			1060					1065					1070		
Glu	Arg	Tyr	Lys	Ile	Gln	Ser	Tyr	Ser	Glu	Tyr	Leu	Lys	Asp	Phe	Trp
		1075					1080					1085			
Gly	Asn	Pro	Leu	Met	Tyr	Asn	Lys	Glu	Tyr	Tyr	Met	Phe	Asn	Ala	Gly
	1090					1095					1100				
Asn	Lys	Asn	Ser	Tyr	Ile	Lys	Leu	Lys	Lys	Asp	Ser	Pro	Val	Gly	Glu
1105					1110					1115					1120
Ile	Leu	Thr	Arg	Ser	Lys	Tyr	Asn	Gln	Asn	Ser	Lys	Tyr	Ile	Asn	Tyr
			1125					1130					1135		
Arg	Asp	Leu	Tyr	Ile	Gly	Glu	Lys	Phe	Ile	Ile	Arg	Arg	Lys	Ser	Asn
		1140						1145					1150		
Ser	Gln	Ser	Ile	Asn	Asp	Asp	Ile	Val	Arg	Lys	Glu	Asp	Tyr	Ile	Tyr
		1155					1160				1165				
Leu	Asp	Phe	Phe	Asn	Leu	Asn	Gln	Glu	Trp	Arg	Val	Tyr	Thr	Tyr	Lys
	1170					1175					1180				
Tyr	Phe	Lys	Lys	Glu	Glu	Glu	Lys	Leu	Phe	Leu	Ala	Pro	Ile	Ser	Asp
1185				1190				1195							1200
Ser	Asp	Glu	Phe	Tyr	Asn	Thr	Ile	Gln	Ile	Lys	Glu	Tyr	Asp	Glu	Gln
			1205					1210					1215		
Pro	Thr	Tyr	Ser	Cys	Gln	Leu	Leu	Phe	Lys	Lys	Asp	Glu	Glu	Ser	Thr
		1220						1225				1230			
Asp	Glu	Ile	Gly	Leu	Ile	Gly	Ile	His	Arg	Phe	Tyr	Glu	Ser	Gly	Ile
	1235					1240					1245				
Val	Phe	Glu	Glu	Tyr	Lys	Asp	Tyr	Phe	Cys	Ile	Ser	Lys	Trp	Tyr	Leu
	1250					1255					1260				
Lys	Glu	Val	Lys	Arg	Lys	Pro	Tyr	Asn	Leu	Lys	Leu	Gly	Cys	Asn	Trp
1265				1270				1275							1280
Gln	Phe	Ile	Pro	Lys	Asp	Glu	Gly	Trp	Thr	Glu					
			1285					1290							

<210> 3

<211> 1291

<212> PRT

<213> Clostridium botulinum Serotype C1

<220>

<221> DOMAIN

<222> (1) ... (449)

<223> Light chain comprising the enzymatic domain.

<221> DOMAIN

<222> (450) ... (855)

<223> Amino-terminal half of heavy chain comprising the translocation domain.

<221> DOMAIN

<222> (856) . . . (1291)

<223> Carboxyl-terminal half of heavy chain comprising the binding domain.

<400> 3

Met	Pro	Ile	Thr	Ile	Asn	Asn	Phe	Asn	Tyr	Ser	Asp	Pro	Val	Asp	Asn
1				5					10					15	
Lys	Asn	Ile	Leu	Tyr	Leu	Asp	Thr	His	Leu	Asn	Thr	Leu	Ala	Asn	Glu

Li *et al.*, Degradable Clostridial Toxins[illegible]

Li et al., Degradable Clostridial Toxins

Val Asp Gln Val Ile Leu Ser Lys Asn Thr Ser Glu His Gly Gln Leu
 500 505 510
 Asp Leu Leu Tyr Pro Ser Ile Asp Ser Glu Ser Glu Ile Leu Pro Gly
 515 520 525
 Glu Asn Gln Val Phe Tyr Asp Asn Arg Thr Gln Asn Val Asp Tyr Leu
 530 535 540
 Asn Ser Tyr Tyr Tyr Leu Glu Ser Gln Lys Leu Ser Asp Asn Val Glu
 545 550 555 560
 Asp Phe Thr Phe Thr Arg Ser Ile Glu Glu Ala Leu Asp Asn Ser Ala
 565 570 575
 Lys Val Tyr Thr Tyr Phe Pro Thr Leu Ala Asn Lys Val Asn Ala Gly
 580 585 590
 Val Gln Gly Gly Leu Phe Leu Met Trp Ala Asn Asp Val Val Glu Asp
 595 600 605
 Phe Thr Thr Asn Ile Leu Arg Lys Asp Thr Leu Asp Lys Ile Ser Asp
 610 615 620
 Val Ser Ala Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Ser Asn
 625 630 635 640
 Ser Val Arg Arg Gly Asn Phe Thr Glu Ala Phe Ala Val Thr Gly Val
 645 650 655
 Thr Ile Leu Leu Glu Ala Phe Pro Glu Phe Thr Ile Pro Ala Leu Gly
 660 665 670
 Ala Phe Val Ile Tyr Ser Lys Val Gln Glu Arg Asn Glu Ile Ile Lys
 675 680 685
 Thr Ile Asp Asn Cys Leu Glu Gln Arg Ile Lys Arg Trp Lys Asp Ser
 690 695 700
 Tyr Glu Trp Met Met Gly Thr Trp Leu Ser Arg Ile Ile Thr Gln Phe
 705 710 715 720
 Asn Asn Ile Ser Tyr Gln Met Tyr Asp Ser Leu Asn Tyr Gln Ala Gly
 725 730 735
 Ala Ile Lys Ala Lys Ile Asp Leu Glu Tyr Lys Lys Tyr Ser Gly Ser
 740 745 750
 Asp Lys Glu Asn Ile Lys Ser Gln Val Glu Asn Leu Lys Asn Ser Leu
 755 760 765
 Asp Val Lys Ile Ser Glu Ala Met Asn Asn Ile Asn Lys Phe Ile Arg
 770 775 780
 Glu Cys Ser Val Thr Tyr Leu Phe Lys Asn Met Leu Pro Lys Val Ile
 785 790 795 800
 Asp Glu Leu Asn Glu Phe Asp Arg Asn Thr Lys Ala Lys Leu Ile Asn
 805 810 815
 Leu Ile Asp Ser His Asn Ile Ile Leu Val Gly Glu Val Asp Lys Leu
 820 825 830
 Lys Ala Lys Val Asn Asn Ser Phe Gln Asn Thr Ile Pro Phe Asn Ile
 835 840 845
 Phe Ser Tyr Thr Asn Asn Ser Leu Leu Lys Asp Ile Ile Asn Glu Tyr
 850 855 860
 Phe Asn Asn Ile Asn Asp Ser Lys Ile Leu Ser Leu Gln Asn Arg Lys
 865 870 875 880
 Asn Thr Leu Val Asp Thr Ser Gly Tyr Asn Ala Glu Val Ser Glu Glu
 885 890 895
 Gly Asp Val Gln Leu Asn Pro Ile Phe Pro Phe Asp Phe Lys Leu Gly
 900 905 910
 Ser Ser Gly Glu Asp Arg Gly Lys Val Ile Val Thr Gln Asn Glu Asn
 915 920 925
 Ile Val Tyr Asn Ser Met Tyr Glu Ser Phe Ser Ile Ser Phe Trp Ile
 930 935 940
 Arg Ile Asn Lys Trp Val Ser Asn Leu Pro Gly Tyr Thr Ile Ile Asp
 945 950 955 960
 Ser Val Lys Asn Asn Ser Gly Trp Ser Ile Gly Ile Ile Ser Asn Phe

Li *et al.*, Degradable Clostridial Toxins

				965					970					975	
Leu	Val	Phe	Thr	Leu	Lys	Gln	Asn	Glu	Asp	Ser	Glu	Gln	Ser	Ile	Asn
				980					985					990	
Phe	Ser	Tyr	Asp	Ile	Ser	Asn	Asn	Ala	Pro	Gly	Tyr	Asn	Lys	Trp	Phe
				995				1000						1005	
Phe	Val	Thr	Val	Thr	Asn	Asn	Met	Met	Gly	Asn	Met	Lys	Ile	Tyr	Ile
				1010				1015						1020	
Asn	Gly	Lys	Leu	Ile	Asp	Thr	Ile	Lys	Val	Lys	Glu	Leu	Thr	Gly	Ile
				1025				1030						1035	
Asn	Phe	Ser	Lys	Thr	Ile	Thr	Phe	Glu	Ile	Asn	Lys	Ile	Pro	Asp	Thr
				1045					1050					1055	
Gly	Leu	Ile	Thr	Ser	Asp	Ser	Asp	Asn	Ile	Asn	Met	Trp	Ile	Arg	Asp
				1060					1065					1070	
Phe	Tyr	Ile	Phe	Ala	Lys	Glu	Leu	Asp	Gly	Lys	Asp	Ile	Asn	Ile	Leu
				1075					1080					1085	
Phe	Asn	Ser	Leu	Gln	Tyr	Thr	Asn	Val	Val	Lys	Asp	Tyr	Trp	Gly	Asn
				1090					1095					1100	
Asp	Leu	Arg	Tyr	Asn	Lys	Glu	Tyr	Tyr	Met	Val	Asn	Ile	Asp	Tyr	Leu
				1105					1110					1115	
Asn	Arg	Tyr	Met	Tyr	Ala	Asn	Ser	Arg	Gln	Ile	Val	Phe	Asn	Thr	Arg
				1125					1130					1135	
Arg	Asn	Asn	Asn	Asp	Phe	Asn	Glu	Gly	Tyr	Lys	Ile	Ile	Ile	Lys	Arg
				1140					1145					1150	
Ile	Arg	Gly	Asn	Thr	Asn	Asp	Thr	Arg	Val	Arg	Gly	Gly	Asp	Ile	Leu
				1155					1160					1165	
Tyr	Phe	Asp	Met	Thr	Ile	Asn	Asn	Lys	Ala	Tyr	Asn	Leu	Phe	Met	Lys
				1170					1175					1180	
Asn	Glu	Thr	Met	Tyr	Ala	Asp	Asn	His	Ser	Thr	Glu	Asp	Ile	Tyr	Ala
				1185					1190					1195	
Ile	Gly	Leu	Arg	Glu	Gln	Thr	Lys	Asp	Ile	Asn	Asp	Asn	Ile	Ile	Phe
				1205					1210					1215	
Gln	Ile	Gln	Pro	Met	Asn	Asn	Thr	Tyr	Tyr	Tyr	Ala	Ser	Gln	Ile	Phe
				1220					1225					1230	
Lys	Ser	Asn	Phe	Asn	Gly	Glu	Asn	Ile	Ser	Gly	Ile	Cys	Ser	Ile	Gly
				1235					1240					1245	
Thr	Tyr	Arg	Phe	Arg	Leu	Gly	Gly	Asp	Trp	Tyr	Arg	His	Asn	Tyr	Leu
				1250					1255					1260	
Val	Pro	Thr	Val	Lys	Gln	Gly	Asn	Tyr	Ala	Ser	Leu	Leu	Glu	Ser	Thr
				1265					1270					1275	
Ser	Thr	His	Trp	Gly	Phe	Val	Pro	Val	Ser	Glu					1280
				1285					1290						

<210> 4

<211> 1276

<212> PRT

<213> Clostridium botulinum Serotype D

<220>

<221> DOMAIN

<222> (1)...(442)

<223> Light chain comprising the enzymatic domain.

<221> DOMAIN

<222> (443)...(851)

<223> Amino-terminal half of heavy chain comprising the translocation domain.

<221> DOMAIN

Li *et al.*, Degradable Clostridial Toxins

<222> (852)...(1276)

<223> Carboxyl-terminal half of heavy chain comprising the binding domain.

<400> 4

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Met Thr Trp Pro Val Lys Asp Phe Asn Tyr Ser Asp Pro Val Asn Asp
 1           5           10           15
Asn Asp Ile Leu Tyr Leu Arg Ile Pro Gln Asn Lys Leu Ile Thr Thr
          20           25           30
Pro Val Lys Ala Phe Met Ile Thr Gln Asn Ile Trp Val Ile Pro Glu
          35           40           45
Arg Phe Ser Ser Asp Thr Asn Pro Ser Leu Ser Lys Pro Pro Arg Pro
          50           55           60
Thr Ser Lys Tyr Gln Ser Tyr Tyr Asp Pro Ser Tyr Leu Ser Thr Asp
65           70           75           80
Glu Gln Lys Asp Thr Phe Leu Lys Gly Ile Ile Lys Leu Phe Lys Arg
          85           90           95
Ile Asn Glu Arg Asp Ile Gly Lys Lys Leu Ile Asn Tyr Leu Val Val
          100          105          110
Gly Ser Pro Phe Met Gly Asp Ser Ser Thr Pro Glu Asp Thr Phe Asp
          115          120          125
Phe Thr Arg His Thr Thr Asn Ile Ala Val Glu Lys Phe Glu Asn Gly
          130          135          140
Ser Trp Lys Val Thr Asn Ile Ile Thr Pro Ser Val Leu Ile Phe Gly
145          150          155          160
Pro Leu Pro Asn Ile Leu Asp Tyr Thr Ala Ser Leu Thr Leu Gln Gly
          165          170          175
Gln Gln Ser Asn Pro Ser Phe Glu Gly Phe Gly Thr Leu Ser Ile Leu
          180          185          190
Lys Val Ala Pro Glu Phe Leu Leu Thr Phe Ser Asp Val Thr Ser Asn
          195          200          205
Gln Ser Ser Ala Val Leu Gly Lys Ser Ile Phe Cys Met Asp Pro Val
          210          215          220
Ile Ala Leu Met His Glu Leu Thr His Ser Leu His Gln Leu Tyr Gly
225          230          235          240
Ile Asn Ile Pro Ser Asp Lys Arg Ile Arg Pro Gln Val Ser Glu Gly
          245          250          255
Phe Phe Ser Gln Asp Gly Pro Asn Val Gln Phe Glu Glu Leu Tyr Thr
          260          265          270
Phe Gly Gly Leu Asp Val Glu Ile Ile Pro Gln Ile Glu Arg Ser Gln
          275          280          285
Leu Arg Glu Lys Ala Leu Gly His Tyr Lys Asp Ile Ala Lys Arg Leu
290          295          300
Asn Asn Ile Asn Lys Thr Ile Pro Ser Ser Trp Ile Ser Asn Ile Asp
305          310          315          320
Lys Tyr Lys Lys Ile Phe Ser Glu Lys Tyr Asn Phe Asp Lys Asp Asn
          325          330          335
Thr Gly Asn Phe Val Val Asn Ile Asp Lys Phe Asn Ser Leu Tyr Ser
          340          345          350
Asp Leu Thr Asn Val Met Ser Glu Val Val Tyr Ser Ser Gln Tyr Asn
          355          360          365
Val Lys Asn Arg Thr His Tyr Phe Ser Arg His Tyr Leu Pro Val Phe
          370          375          380
Ala Asn Ile Leu Asp Asp Asn Ile Tyr Thr Ile Arg Asp Gly Phe Asn
385          390          395          400
Leu Thr Asn Lys Gly Phe Asn Ile Glu Asn Ser Gly Gln Asn Ile Glu
          405          410          415
Arg Asn Pro Ala Leu Gln Lys Leu Ser Ser Glu Ser Val Val Asp Leu

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Li et al., Degradable Clostridial Toxins

			420					425					430				
Phe	Thr	Lys	Val	Cys	Leu	Arg	Leu	Thr	Lys	Asn	Ser	Arg	Asp	Asp	Ser		
		435					440					445					
Thr	Cys	Ile	Lys	Val	Lys	Asn	Asn	Arg	Leu	Pro	Tyr	Val	Ala	Asp	Lys		
	450					455					460						
Asp	Ser	Ile	Ser	Gln	Glu	Ile	Phe	Glu	Asn	Lys	Ile	Ile	Thr	Asp	Glu		
465					470					475					480		
Thr	Asn	Val	Gln	Asn	Tyr	Ser	Asp	Lys	Phe	Ser	Leu	Asp	Glu	Ser	Ile		
			485					490						495			
Leu	Asp	Gly	Gln	Val	Pro	Ile	Asn	Pro	Glu	Ile	Val	Asp	Pro	Leu	Leu		
		500					505					510					
Pro	Asn	Val	Asn	Met	Glu	Pro	Leu	Asn	Leu	Pro	Gly	Glu	Glu	Ile	Val		
	515					520					525						
Phe	Tyr	Asp	Asp	Ile	Thr	Lys	Tyr	Val	Asp	Tyr	Leu	Asn	Ser	Tyr	Tyr		
	530					535				540							
Tyr	Leu	Glu	Ser	Gln	Lys	Leu	Ser	Asn	Asn	Val	Glu	Asn	Ile	Thr	Leu		
545					550				555						560		
Thr	Thr	Ser	Val	Glu	Glu	Ala	Leu	Gly	Tyr	Ser	Asn	Lys	Ile	Tyr	Thr		
			565					570						575			
Phe	Leu	Pro	Ser	Leu	Ala	Glu	Lys	Val	Asn	Lys	Gly	Val	Gln	Ala	Gly		
			580					585					590				
Leu	Phe	Leu	Asn	Trp	Ala	Asn	Glu	Val	Val	Glu	Asp	Phe	Thr	Thr	Asn		
	595					600					605						
Ile	Met	Lys	Lys	Asp	Thr	Leu	Asp	Lys	Ile	Ser	Asp	Val	Ser	Val	Ile		
	610					615					620						
Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Ser	Ala	Leu	Arg		
625					630					635					640		
Gly	Asn	Phe	Asn	Gln	Ala	Phe	Ala	Thr	Ala	Gly	Val	Ala	Phe	Leu	Leu		
			645					650						655			
Glu	Gly	Phe	Pro	Glu	Phe	Thr	Ile	Pro	Ala	Leu	Gly	Val	Phe	Thr	Phe		
		660					665					670					
Tyr	Ser	Ser	Ile	Gln	Glu	Arg	Glu	Lys	Ile	Ile	Lys	Thr	Ile	Glu	Asn		
	675				680						685						
Cys	Leu	Glu	Gln	Arg	Val	Lys	Arg	Trp	Lys	Asp	Ser	Tyr	Gln	Trp	Met		
	690				695					700							
Val	Ser	Asn	Trp	Leu	Ser	Arg	Ile	Thr	Thr	Gln	Phe	Asn	His	Ile	Asn		
705					710					715					720		
Tyr	Gln	Met	Tyr	Asp	Ser	Leu	Ser	Tyr	Gln	Ala	Asp	Ala	Ile	Lys	Ala		
			725					730						735			
Lys	Ile	Asp	Leu	Glu	Tyr	Lys	Lys	Tyr	Ser	Gly	Ser	Asp	Lys	Glu	Asn		
		740						745					750				
Ile	Lys	Ser	Gln	Val	Glu	Asn	Leu	Lys	Asn	Ser	Leu	Asp	Val	Lys	Ile		
	755					760					765						
Ser	Glu	Ala	Met	Asn	Asn	Ile	Asn	Lys	Phe	Ile	Arg	Glu	Cys	Ser	Val		
	770				775					780							
Thr	Tyr	Leu	Phe	Lys	Asn	Met	Leu	Pro	Lys	Val	Ile	Asp	Glu	Leu	Asn		
785					790					795					800		
Lys	Phe	Asp	Leu	Arg	Thr	Lys	Thr	Glu	Leu	Ile	Asn	Leu	Ile	Asp	Ser		
			805					810						815			
His	Asn	Ile	Ile	Leu	Val	Gly	Glu	Val	Asp	Arg	Leu	Lys	Ala	Lys	Val		
		820						825					830				
Asn	Glu	Ser	Phe	Glu	Asn	Thr	Met	Pro	Phe	Asn	Ile	Phe	Ser	Tyr	Thr		
	835					840						845					
Asn	Asn	Ser	Leu	Leu	Lys	Asp	Ile	Ile	Asn	Glu	Tyr	Phe	Asn	Ser	Ile		
	850				855					860							
Asn	Asp	Ser	Lys	Ile	Leu	Ser	Leu	Gln	Asn	Lys	Lys	Asn	Ala	Leu	Val		
865					870					875					880		
Asp	Thr	Ser	Gly	Tyr	Asn	Ala	Glu	Val	Arg	Val	Gly	Asp	Asn	Val	Gln		

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										885					890					895					
Leu	Asn	Thr	Ile	Tyr	Thr	Asn	Asp	Phe	Lys	Leu	Ser	Ser	Ser	Gly	Asp										
			900				905						910												
Lys	Ile	Ile	Val	Asn	Leu	Asn	Asn	Asn	Ile	Leu	Tyr	Ser	Ala	Ile	Tyr										
			915				920						925												
Glu	Asn	Ser	Ser	Val	Ser	Phe	Trp	Ile	Lys	Ile	Ser	Lys	Asp	Leu	Thr										
			930				935						940												
Asn	Ser	His	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Ser	Ile	Glu	Gln	Asn	Ser										
			945				950						955			960									
Gly	Trp	Lys	Leu	Cys	Ile	Arg	Asn	Gly	Asn	Ile	Glu	Trp	Ile	Leu	Gln										
															895										
										890					895										
Asp	Val	Asn	Arg	Lys	Tyr	Lys	Ser	Leu	Ile	Phe	Asp	Tyr	Ser	Glu	Ser										
			980				985						990												
Leu	Ser	His	Thr	Gly	Tyr	Thr	Asn	Lys	Trp	Phe	Phe	Val	Thr	Ile	Thr										
			995				1000						1005												
Asn	Asn	Ile	Met	Gly	Tyr	Met	Lys	Leu	Tyr	Ile	Asn	Gly	Glu	Leu	Lys										
			1010				1015						1020												
Gln	Ser	Gln	Lys	Ile	Glu	Asp	Leu	Asp	Glu	Val	Lys	Leu	Asp	Lys	Thr										
			1025				1030						1035			1040									
Ile	Val	Phe	Gly	Ile	Asp	Glu	Asn	Ile	Asp	Glu	Asn	Gln	Met	Leu	Trp										
			1045				1050						1055												
Ile	Arg	Asp	Phe	Asn	Ile	Phe	Ser	Lys	Glu	Leu	Ser	Asn	Glu	Asp	Ile										
			1060				1065						1070												
Asn	Ile	Val	Tyr	Glu	Gly	Gln	Ile	Leu	Arg	Asn	Val	Ile	Lys	Asp	Tyr										
			1075				1080						1085												
Trp	Gly	Asn	Pro	Leu	Lys	Phe	Asp	Thr	Glu	Tyr	Tyr	Ile	Ile	Asn	Asp										
			1090				1095						1100												
Asn	Tyr	Ile	Asp	Arg	Tyr	Ile	Ala	Pro	Glu	Ser	Asn	Val	Leu	Val	Leu										
			1105				1110						1115			1120									
Val	Gln	Tyr	Pro	Asp	Arg	Ser	Lys	Leu	Tyr	Thr	Gly	Asn	Pro	Ile	Thr										
			1125				1130						1135												
Ile	Lys	Ser	Val	Ser	Asp	Lys	Asn	Pro	Tyr	Ser	Arg	Ile	Leu	Asn	Gly										
			1140				1145						1150												
Asp	Asn	Ile	Leu	His	Met	Leu	Tyr	Asn	Ser	Arg	Lys	Tyr	Met	Ile											
			1155				1160						1165												
Ile	Arg	Asp	Thr	Asp	Thr	Ile	Tyr	Ala	Thr	Gln	Gly	Gly	Glu	Cys	Ser										
			1170				1175						1180												
Gln	Asn	Cys	Val	Tyr	Ala	Leu	Lys	Leu	Gln	Ser	Asn	Leu	Gly	Asn	Tyr										
			1185				1190						1195			1200									
Gly	Ile	Gly	Ile	Phe	Ser	Ile	Lys	Asn	Ile	Val	Ser	Lys	Asn	Lys	Tyr										
			1205				1210						1215												
Cys	Ser	Gln	Ile	Phe	Ser	Ser	Phe	Arg	Glu	Asn	Thr	Met	Leu	Leu	Ala										
			1220				1225						1230												

<210> 5

<211> 1252

<212> PRT

<213> Clostridium botulinum Serotype E

<220>

<221> DOMAIN

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<222> (1)...(422)

<223> Light chain comprising the enzymatic domain.

<221> DOMAIN

<222> (423)...(834)

<223> Amino-terminal half of heavy chain comprising the translocation domain.

<221> DOMAIN

<222> (835)...(1252)

<223> Carboxyl-terminal half of heavy chain comprising the binding domain.

<400> 5

Met	Pro	Lys	Ile	Asn	Ser	Phe	Asn	Tyr	Asn	Asp	Pro	Val	Asn	Asp	Arg	1	5	10	15
Thr	Ile	Leu	Tyr	Ile	Lys	Pro	Gly	Gly	Cys	Gln	Glu	Phe	Tyr	Lys	Ser	20	25	30	
Phe	Asn	Ile	Met	Lys	Asn	Ile	Trp	Ile	Ile	Pro	Glu	Arg	Asn	Val	Ile	35	40	45	
Gly	Thr	Thr	Pro	Gln	Asp	Phe	His	Pro	Pro	Thr	Ser	Leu	Lys	Asn	Gly	50	55	60	
Asp	Ser	Ser	Tyr	Tyr	Asp	Pro	Asn	Tyr	Leu	Gln	Ser	Asp	Glu	Glu	Lys	65	70	75	80
Asp	Arg	Phe	Leu	Lys	Ile	Val	Thr	Lys	Ile	Phe	Asn	Arg	Ile	Asn	Asn	85	90	95	
Asn	Leu	Ser	Gly	Ile	Leu	Leu	Glu	Glu	Leu	Ser	Lys	Ala	Asn	Pro		100	105	110	
Tyr	Leu	Gly	Asn	Asp	Asn	Thr	Pro	Asp	Asn	Gln	Phe	His	Ile	Gly	Asp	115	120	125	
Ala	Ser	Ala	Val	Glu	Ile	Lys	Phe	Ser	Asn	Gly	Ser	Gln	Asp	Ile	Leu	130	135	140	
Leu	Pro	Asn	Val	Ile	Ile	Met	Gly	Ala	Glu	Pro	Asp	Leu	Phe	Glu	Thr	145	150	155	160
Asn	Ser	Ser	Asn	Ile	Ser	Leu	Arg	Asn	Asn	Tyr	Met	Pro	Ser	Asn	His	165	170	175	
Gly	Phe	Gly	Ser	Ile	Ala	Ile	Val	Thr	Phe	Ser	Pro	Glu	Tyr	Ser	Phe	180	185	190	
Arg	Phe	Asn	Asp	Asn	Ser	Met	Asn	Glu	Phe	Ile	Gln	Asp	Pro	Ala	Leu	195	200	205	
Thr	Leu	Met	His	Glu	Leu	Ile	His	Ser	Leu	His	Gly	Leu	Tyr	Gly	Ala	210	215	220	
Lys	Gly	Ile	Thr	Thr	Lys	Tyr	Thr	Ile	Thr	Gln	Lys	Gln	Asn	Pro	Leu	225	230	235	240
Ile	Thr	Asn	Ile	Arg	Gly	Thr	Asn	Ile	Glu	Glu	Phe	Leu	Thr	Phe	Gly	245	250	255	
Gly	Thr	Asp	Leu	Asn	Ile	Ile	Thr	Ser	Ala	Gln	Ser	Asn	Asp	Ile	Tyr	260	265	270	
Thr	Asn	Leu	Leu	Ala	Asp	Tyr	Lys	Lys	Ile	Ala	Ser	Lys	Leu	Ser	Lys	275	280	285	
Val	Gln	Val	Ser	Asn	Pro	Leu	Leu	Asn	Pro	Tyr	Lys	Asp	Val	Phe	Glu	290	295	300	
Ala	Lys	Tyr	Gly	Leu	Asp	Lys	Asp	Ala	Ser	Gly	Ile	Tyr	Ser	Val	Asn	305	310	315	320
Ile	Asn	Lys	Phe	Asn	Asp	Ile	Phe	Lys	Lys	Leu	Tyr	Ser	Phe	Thr	Glu	325	330	335	
Phe	Asp	Leu	Ala	Thr	Lys	Phe	Gln	Val	Lys	Cys	Arg	Gln	Thr	Tyr	Ile	340	345	350	

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Gly Gln Tyr Lys Tyr Phe Lys Leu Ser Asn Leu Leu Asn Asp Ser Ile
 355 360 365
 Tyr Asn Ile Ser Glu Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe
 370 375 380
 Arg Gly Gln Asn Ala Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr
 385 390 395 400
 Gly Arg Gly Leu Val Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val
 405 410 415
 Ser Val Lys Gly Ile Arg Lys Ser Ile Cys Ile Glu Ile Asn Asn Gly
 420 425 430
 Glu Leu Phe Phe Val Ala Ser Glu Asn Ser Tyr Asn Asp Asp Asn Ile
 435 440 445
 Asn Thr Pro Lys Glu Ile Asp Asp Thr Val Thr Ser Asn Asn Asn Tyr
 450 455 460
 Glu Asn Asp Leu Asp Gln Val Ile Leu Asn Phe Asn Ser Glu Ser Ala
 465 470 475 480
 Pro Gly Leu Ser Asp Glu Lys Leu Asn Leu Thr Ile Gln Asn Asp Ala
 485 490 495
 Tyr Ile Pro Lys Tyr Asp Ser Asn Gly Thr Ser Asp Ile Glu Gln His
 500 505 510
 Asp Val Asn Glu Leu Asn Val Phe Phe Tyr Leu Asp Ala Gln Lys Val
 515 520 525
 Pro Glu Gly Glu Asn Asn Val Asn Leu Thr Ser Ser Ile Asp Thr Ala
 530 535 540
 Leu Leu Glu Gln Pro Lys Ile Tyr Thr Phe Phe Ser Ser Glu Phe Ile
 545 550 555 560
 Asn Asn Val Asn Lys Pro Val Gln Ala Ala Leu Phe Val Ser Trp Ile
 565 570 575
 Gln Gln Val Leu Val Asp Phe Thr Thr Glu Ala Asn Gln Lys Ser Thr
 580 585 590
 Val Asp Lys Ile Ala Asp Ile Ser Ile Val Val Pro Tyr Ile Gly Leu
 595 600 605
 Ala Leu Asn Ile Gly Asn Glu Ala Gln Lys Gly Asn Phe Lys Asp Ala
 610 615 620
 Leu Glu Leu Leu Gly Ala Gly Ile Leu Leu Glu Phe Glu Pro Glu Leu
 625 630 635 640
 Leu Ile Pro Thr Ile Leu Val Phe Thr Ile Lys Ser Phe Leu Gly Ser
 645 650 655
 Ser Asp Asn Lys Asn Lys Val Ile Lys Ala Ile Asn Asn Ala Leu Lys
 660 665 670
 Glu Arg Asp Glu Lys Trp Lys Glu Val Tyr Ser Phe Ile Val Ser Asn
 675 680 685
 Trp Met Thr Lys Ile Asn Thr Gln Phe Asn Lys Arg Lys Glu Gln Met
 690 695 700
 Tyr Gln Ala Leu Gln Asn Gln Val Asn Ala Ile Lys Thr Ile Ile Glu
 705 710 715 720
 Ser Lys Tyr Asn Ser Tyr Thr Leu Glu Glu Lys Asn Glu Leu Thr Asn
 725 730 735
 Lys Tyr Asp Ile Lys Gln Ile Glu Asn Glu Leu Asn Gln Lys Val Ser
 740 745 750
 Ile Ala Met Asn Asn Ile Asp Arg Phe Leu Thr Glu Ser Ser Ile Ser
 755 760 765
 Tyr Leu Met Lys Leu Ile Asn Glu Val Lys Ile Asn Lys Leu Arg Glu
 770 775 780
 Tyr Asp Glu Asn Val Lys Thr Tyr Leu Leu Asn Tyr Ile Ile Gln His
 785 790 795 800
 Gly Ser Ile Leu Gly Glu Ser Gln Gln Glu Leu Asn Ser Met Val Thr
 805 810 815
 Asp Thr Leu Asn Asn Ser Ile Pro Phe Lys Leu Ser Ser Tyr Thr Asp

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<212> PRT

<213> Clostridium botulinum Serotype F

<220>

<221> DOMAIN

<222> (1)...(436)

<223> Light chain comprising the enzymatic domain.

<221> DOMAIN

<222> (437)...(852)

<223> Amino-terminal half of heavy chain comprising the translocation domain.

<221> DOMAIN

<222> (853)...(1274)

<223> Carboxyl-terminal half of heavy chain comprising the binding domain.

<400> 6

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Met Pro Val Ala Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asp
 1           5           10           15
Asp Thr Ile Leu Tyr Met Gln Ile Pro Tyr Glu Glu Lys Ser Lys Lys
      20           25           30
Tyr Tyr Lys Ala Phe Glu Ile Met Arg Asn Val Trp Ile Ile Pro Glu
      35           40           45
Arg Asn Thr Ile Gly Thr Asn Pro Ser Asp Phe Asp Pro Pro Ala Ser
      50           55           60
Leu Lys Asn Gly Ser Ser Ala Tyr Tyr Asp Pro Asn Tyr Leu Thr Thr
      65           70           75           80
Asp Ala Glu Lys Asp Arg Tyr Leu Lys Thr Thr Ile Lys Leu Phe Lys
      85           90           95
Arg Ile Asn Ser Asn Pro Ala Gly Lys Val Leu Leu Gln Glu Ile Ser
      100          105          110
Tyr Ala Lys Pro Tyr Leu Gly Asn Asp His Thr Pro Ile Asp Glu Phe
      115          120          125
Ser Pro Val Thr Arg Thr Thr Ser Val Asn Ile Lys Leu Ser Thr Asn
      130          135          140
Val Glu Ser Ser Met Leu Leu Asn Leu Leu Val Leu Gly Ala Gly Pro
      145          150          155          160
Asp Ile Phe Glu Ser Cys Cys Tyr Pro Val Arg Lys Leu Ile Asp Pro
      165          170          175
Asp Val Val Tyr Asp Pro Ser Asn Tyr Gly Phe Gly Ser Ile Asn Ile
      180          185          190
Val Thr Phe Ser Pro Glu Tyr Glu Tyr Thr Phe Asn Asp Ile Ser Gly
      195          200          205
Gly His Asn Ser Ser Thr Glu Ser Phe Ile Ala Asp Pro Ala Ile Ser
      210          215          220
Leu Ala His Glu Leu Ile His Ala Leu His Gly Leu Tyr Gly Ala Arg
      225          230          235          240
Gly Val Thr Tyr Glu Glu Thr Ile Glu Val Lys Gln Ala Pro Leu Met
      245          250          255
Ile Ala Glu Lys Pro Ile Arg Leu Glu Glu Phe Leu Thr Phe Gly Gly
      260          265          270
Gln Asp Leu Asn Ile Ile Thr Ser Ala Met Lys Glu Lys Ile Tyr Asn
      275          280          285
Asn Leu Leu Ala Asn Tyr Glu Lys Ile Ala Thr Arg Leu Ser Glu Val
      290          295          300
Asn Ser Ala Pro Pro Glu Tyr Asp Ile Asn Glu Tyr Lys Asp Tyr Phe
      305          310          315          320

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Gln	Trp	Lys	Tyr	Gly	Leu	Asp	Lys	Asn	Ala	Asp	Gly	Ser	Tyr	Thr	Val
				325					330					335	
Asn	Glu	Asn	Lys	Phe	Asn	Glu	Ile	Tyr	Lys	Lys	Leu	Tyr	Ser	Phe	Thr
			340					345					350		
Glu	Ser	Asp	Leu	Ala	Asn	Lys	Phe	Lys	Val	Lys	Cys	Arg	Asn	Thr	Tyr
		355					360					365			
Phe	Ile	Lys	Tyr	Glu	Phe	Leu	Lys	Val	Pro	Asn	Leu	Leu	Asp	Asp	Asp
	370					375					380				
Ile	Tyr	Thr	Val	Ser	Glu	Gly	Phe	Asn	Ile	Gly	Asn	Leu	Ala	Val	Asn
385					390					395					400
Asn	Arg	Gly	Gln	Ser	Ile	Lys	Leu	Asn	Pro	Lys	Ile	Ile	Asp	Ser	Ile
			405						410					415	
Pro	Asp	Lys	Gly	Leu	Val	Glu	Lys	Ile	Val	Lys	Phe	Cys	Lys	Ser	Val
		420						425					430		
Ile	Pro	Arg	Lys	Gly	Thr	Lys	Ala	Pro	Pro	Arg	Leu	Cys	Ile	Arg	Val
	435						440					445			
Asn	Asn	Ser	Glu	Leu	Phe	Phe	Val	Ala	Ser	Glu	Ser	Ser	Tyr	Asn	Glu
	450					455					460				
Asn	Asp	Ile	Asn	Thr	Pro	Lys	Glu	Ile	Asp	Asp	Thr	Thr	Asn	Leu	Asn
465					470					475					480
Asn	Asn	Tyr	Arg	Asn	Asn	Leu	Asp	Glu	Val	Ile	Leu	Asp	Tyr	Asn	Ser
			485						490					495	
Gln	Thr	Ile	Pro	Gln	Ile	Ser	Asn	Arg	Thr	Leu	Asn	Thr	Leu	Val	Gln
		500						505					510		
Asp	Asn	Ser	Tyr	Val	Pro	Arg	Tyr	Asp	Ser	Asn	Gly	Thr	Ser	Glu	Ile
	515						520					525			
Glu	Glu	Tyr	Asp	Val	Val	Asp	Phe	Asn	Val	Phe	Phe	Tyr	Leu	His	Ala
	530					535					540				
Gln	Lys	Val	Pro	Glu	Gly	Glu	Thr	Asn	Ile	Ser	Leu	Thr	Ser	Ser	Ile
545					550					555					560
Asp	Thr	Ala	Leu	Leu	Glu	Glu	Ser	Lys	Asp	Ile	Phe	Phe	Ser	Ser	Glu
			565						570					575	
Phe	Ile	Asp	Thr	Ile	Asn	Lys	Pro	Val	Asn	Ala	Ala	Leu	Phe	Ile	Asp
		580						585					590		
Trp	Ile	Ser	Lys	Val	Ile	Arg	Asp	Phe	Thr	Thr	Glu	Ala	Thr	Gln	Lys
	595						600					605			
Ser	Thr	Val	Asp	Lys	Ile	Ala	Asp	Ile	Ser	Leu	Ile	Val	Pro	Tyr	Val
	610					615					620				
Gly	Leu	Ala	Leu	Asn	Ile	Ile	Ile	Glu	Ala	Glu	Lys	Gly	Asn	Phe	Glu
625					630					635					640
Glu	Ala	Phe	Glu	Leu	Gly	Val	Gly	Ile	Leu	Leu	Glu	Phe	Val	Pro	
			645					650					655		
Glu	Leu	Thr	Ile	Pro	Val	Ile	Leu	Val	Phe	Thr	Ile	Lys	Ser	Tyr	Ile
		660						665					670		
Asp	Ser	Tyr	Glu	Asn	Lys	Asn	Lys	Ala	Ile	Lys	Ala	Ile	Asn	Asn	Ser
		675					680					685			
Leu	Ile	Glu	Arg	Glu	Ala	Lys	Trp	Lys	Glu	Ile	Tyr	Ser	Trp	Ile	Val
	690					695					700				
Ser	Asn	Trp	Leu	Thr	Arg	Ile	Asn	Thr	Gln	Phe	Asn	Lys	Arg	Lys	Glu
705					710					715					720
Gln	Met	Tyr	Gln	Ala	Leu	Gln	Asn	Gln	Val	Asp	Ala	Ile	Lys	Thr	Ala
			725						730					735	
Ile	Glu	Tyr	Lys	Tyr	Asn	Asn	Tyr	Thr	Ser	Asp	Glu	Lys	Asn	Arg	Leu
		740						745					750		
Glu	Ser	Glu	Tyr	Asn	Ile	Asn	Asn	Ile	Glu	Glu	Glu	Leu	Asn	Lys	Lys
		755					760					765			
Val	Ser	Leu	Ala	Met	Lys	Asn	Ile	Glu	Arg	Phe	Met	Thr	Glu	Ser	Ser
	770					775					780				
Ile	Ser	Tyr	Leu	Met	Lys	Leu	Ile	Asn	Glu	Ala	Lys	Val	Gly	Lys	Leu

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785		790		795		800
Lys Lys Tyr Asp	Asn His Val Lys Ser	Asp Leu Leu Asn Tyr	Ile Leu			
	805	810	815			
Asp His Arg Ser	Ile Leu Gly Glu Gln Thr	Asn Glu Leu Ser	Asp Leu			
	820	825	830			
Val Thr Ser Thr	Leu Asn Ser Ser	Ile Pro Phe Glu Leu	Ser Ser Tyr			
	835	840	845			
Thr Asn Asp Lys	Ile Leu Ile Ile Tyr	Phe Asn Arg Leu	Tyr Lys Lys			
	850	855	860			
Ile Lys Asp Ser	Ser Ile Leu Asp Met Arg	Tyr Glu Asn Asn	Lys Phe			
865	870	875	880			
Ile Asp Ile Ser	Gly Tyr Gly Ser Asn	Ile Ser Ile Asn	Gly Asn Val			
	885	890	895			
Tyr Ile Tyr Ser	Thr Asn Arg Asn Gln	Phe Gly Ile Tyr	Asn Ser Arg			
	900	905	910			
Leu Ser Glu Val	Asn Ile Ala Gln Asn Asn	Asp Ile Ile Tyr	Asn Ser			
	915	920	925			
Arg Tyr Gln Asn	Phe Ser Ile Ser Phe Trp	Val Arg Ile Pro	Lys His			
	930	935	940			
Tyr Lys Pro Met	Asn His Asn Arg Glu Tyr	Thr Ile Ile Asn	Cys Met			
945	950	955	960			
Gly Asn Asn Asn	Ser Gly Trp Lys Ile Ser	Leu Arg Thr Val	Arg Asp			
	965	970	975			
Cys Glu Ile Ile	Trp Thr Leu Gln Asp Thr	Ser Gly Asn Lys	Glu Asn			
	980	985	990			
Leu Ile Phe Arg	Tyr Glu Glu Leu Asn Arg	Ile Ser Asn Tyr	Ile Asn			
	995	1000	1005			
Lys Trp Ile Phe	Val Thr Ile Thr Asn Asn	Arg Leu Gly Asn	Ser Arg			
	1010	1015	1020			
Ile Tyr Ile Asn	Gly Asn Leu Ile Val Glu	Lys Ser Ile Ser	Asn Leu			
1025	1030	1035	1040			
Gly Asp Ile His	Val Ser Asp Asn Ile Leu	Phe Lys Ile Val	Gly Cys			
	1045	1050	1055			
Asp Asp Glu Thr	Tyr Val Gly Ile Arg Tyr	Phe Lys Val Phe	Asn Thr			
	1060	1065	1070			
Glu Leu Asp Lys	Thr Glu Ile Glu Thr Leu Tyr	Ser Asn Glu Pro	Asp			
	1075	1080	1085			
Pro Ser Ile Leu	Lys Asn Tyr Trp Gly Asn Tyr	Leu Leu Tyr Asn	Lys			
	1090	1095	1100			
Lys Tyr Tyr Leu	Phe Asn Leu Leu Arg Lys	Asp Lys Tyr Ile Thr	Leu			
1105	1110	1115	1120			
Asn Ser Gly Ile	Leu Asn Ile Asn Gln Gln	Arg Gly Val Thr	Glu Gly			
	1125	1130	1135			
Ser Val Phe Leu	Asn Tyr Lys Leu Tyr Glu Gly	Val Glu Val Ile Ile				
	1140	1145	1150			
Arg Lys Asn Gly	Pro Ile Asp Ile Ser Asn Thr	Asp Asn Phe Val Arg				
	1155	1160	1165			
Lys Asn Asp Leu	Ala Tyr Ile Asn Val Val Asp	Arg Gly Val Glu Tyr				
	1170	1175	1180			
Arg Leu Tyr Ala	Asp Thr Lys Ser Glu Lys Glu	Lys Ile Ile Arg Thr				
1185	1190	1195	1200			
Ser Asn Leu Asn	Asp Ser Leu Gly Gln Ile Ile	Val Met Asp Ser Ile				
	1205	1210	1215			
Gly Asn Asn Cys	Thr Met Asn Phe Gln Asn Asn	Asn Gly Ser Asn Ile				
	1220	1225	1230			
Gly Leu Leu Gly	Phe His Ser Asn Asn Leu Val	Ala Ser Ser Trp Tyr				
	1235	1240	1245			
Tyr Asn Asn Ile	Arg Arg Asn Thr Ser Ser Asn	Gly Cys Phe Trp Ser				
1250	1255	1260				

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Ser Ile Ser Lys Glu Asn Gly Trp Lys Glu
1265 1270

<210> 7

<211> 1297

<212> PRT

<213> Clostridium botulinum Serotype G

<220>

<221> DOMAIN

<222> (1)...(442)

<223> Light chain comprising the enzymatic domain.

<221> DOMAIN

<222> (443)...(852)

<223> Amino-terminal half of heavy chain comprising the translocation domain.

<221> DOMAIN

<222> (853)...(1297)

<223> Carboxyl-terminal half of heavy chain comprising the binding domain.

<400> 7

Met	Pro	Val	Asn	Ile	Lys	Asn	Phe	Asn	Tyr	Asn	Asp	Pro	Ile	Asn	Asn
1			5					10						15	
Asp	Asp	Ile	Ile	Met	Met	Glu	Pro	Phe	Asn	Asp	Pro	Gly	Pro	Gly	Thr
		20					25					30			
Tyr	Tyr	Lys	Ala	Phe	Arg	Ile	Ile	Asp	Arg	Ile	Trp	Ile	Val	Pro	Glu
		35				40					45				
Arg	Phe	Thr	Tyr	Gly	Phe	Gln	Pro	Asp	Gln	Phe	Asn	Ala	Ser	Thr	Gly
	50					55				60					
Val	Phe	Ser	Lys	Asp	Val	Tyr	Glu	Tyr	Tyr	Asp	Pro	Thr	Tyr	Leu	Lys
65				70					75					80	
Thr	Asp	Ala	Glu	Lys	Asp	Lys	Phe	Leu	Lys	Thr	Met	Ile	Lys	Leu	Phe
			85					90					95		
Asn	Arg	Ile	Asn	Ser	Lys	Pro	Ser	Gly	Gln	Arg	Leu	Leu	Asp	Met	Ile
			100					105					110		
Val	Asp	Ala	Ile	Pro	Tyr	Leu	Gly	Asn	Ala	Ser	Thr	Pro	Pro	Asp	Lys
		115				120						125			
Phe	Ala	Ala	Asn	Val	Ala	Asn	Val	Ser	Ile	Asn	Lys	Lys	Ile	Ile	Gln
	130					135					140				
Pro	Gly	Ala	Glu	Asp	Gln	Ile	Lys	Gly	Leu	Met	Thr	Asn	Leu	Ile	Ile
145				150					155					160	
Phe	Gly	Pro	Gly	Pro	Val	Leu	Ser	Asp	Asn	Phe	Thr	Asp	Ser	Met	Ile
			165					170						175	
Met	Asn	Gly	His	Ser	Pro	Ile	Ser	Glu	Gly	Phe	Gly	Ala	Arg	Met	Met
			180					185					190		
Ile	Arg	Phe	Cys	Pro	Ser	Cys	Leu	Asn	Val	Phe	Asn	Asn	Val	Gln	Glu
		195				200					205				
Asn	Lys	Asp	Thr	Ser	Ile	Phe	Ser	Arg	Arg	Ala	Tyr	Phe	Ala	Asp	Pro
	210					215					220				
Ala	Leu	Thr	Leu	Met	His	Glu	Leu	Ile	His	Val	Leu	His	Gly	Leu	Tyr
225				230					235					240	
Gly	Ile	Lys	Ile	Ser	Asn	Leu	Pro	Ile	Thr	Pro	Asn	Thr	Lys	Glu	Phe
			245					250						255	
Phe	Met	Gln	His	Ser	Asp	Pro	Val	Gln	Ala	Glu	Glu	Leu	Tyr	Thr	Phe
		260						265					270		

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Gly	Gly	His	Asp	Pro	Ser	Val	Ile	Ser	Pro	Ser	Thr	Asp	Met	Asn	Ile	275	280	285
Tyr	Asn	Lys	Ala	Leu	Gln	Asn	Phe	Gln	Asp	Ile	Ala	Asn	Arg	Leu	Asn	290	295	300
Ile	Val	Ser	Ser	Ala	Gln	Gly	Ser	Gly	Ile	Asp	Ile	Ser	Leu	Tyr	Lys	305	310	315
Gln	Ile	Tyr	Lys	Asn	Lys	Tyr	Asp	Phe	Val	Glu	Asp	Pro	Asn	Gly	Lys	325	330	335
Tyr	Ser	Val	Asp	Lys	Asp	Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Ala	Leu	Met	340	345	350
Phe	Gly	Phe	Thr	Glu	Thr	Asn	Leu	Ala	Gly	Glu	Tyr	Gly	Ile	Lys	Thr	355	360	365
Arg	Tyr	Ser	Tyr	Phe	Ser	Glu	Tyr	Leu	Pro	Pro	Ile	Lys	Thr	Glu	Lys	370	375	380
Leu	Leu	Asp	Asn	Thr	Ile	Tyr	Thr	Gln	Asn	Glu	Gly	Phe	Asn	Ile	Ala	385	390	395
Ser	Lys	Asn	Leu	Lys	Thr	Glu	Phe	Asn	Gly	Gln	Asn	Lys	Ala	Val	Asn	405	410	415
Lys	Glu	Ala	Tyr	Glu	Glu	Ile	Ser	Leu	Glu	His	Leu	Val	Ile	Tyr	Arg	420	425	430
Ile	Ala	Met	Cys	Lys	Pro	Val	Met	Tyr	Lys	Asn	Thr	Gly	Lys	Ser	Glu	435	440	445
Gln	Cys	Ile	Ile	Val	Asn	Asn	Glu	Asp	Leu	Phe	Phe	Ile	Ala	Asn	Lys	450	455	460
Asp	Ser	Phe	Ser	Lys	Asp	Leu	Ala	Lys	Ala	Glu	Thr	Ile	Ala	Tyr	Asn	465	470	475
Thr	Gln	Asn	Asn	Thr	Ile	Glu	Asn	Asn	Phe	Ser	Ile	Asp	Gln	Leu	Ile	485	490	495
Leu	Asp	Asn	Asp	Leu	Ser	Ser	Gly	Ile	Asp	Leu	Pro	Asn	Glu	Asn	Thr	500	505	510
Glu	Pro	Phe	Thr	Asn	Phe	Asp	Asp	Ile	Asp	Ile	Pro	Val	Tyr	Ile	Lys	515	520	525
Gln	Ser	Ala	Leu	Lys	Lys	Ile	Phe	Val	Asp	Gly	Asp	Ser	Leu	Phe	Glu	530	535	540
Tyr	Leu	His	Ala	Gln	Thr	Phe	Pro	Ser	Asn	Ile	Glu	Asn	Leu	Gln	Leu	545	550	555
Thr	Asn	Ser	Leu	Asn	Asp	Ala	Leu	Arg	Asn	Asn	Asn	Lys	Val	Tyr	Thr	565	570	575
Phe	Phe	Ser	Thr	Asn	Leu	Val	Glu	Lys	Ala	Asn	Thr	Val	Val	Gly	Ala	580	585	590
Ser	Leu	Phe	Val	Asn	Trp	Val	Lys	Gly	Val	Ile	Asp	Asp	Phe	Thr	Ser	595	600	605
Glu	Ser	Thr	Gln	Lys	Ser	Thr	Ile	Asp	Lys	Val	Ser	Asp	Val	Ser	Ile	610	615	620
Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Val	Gly	Asn	Glu	Thr	Ala	625	630	635
Lys	Glu	Asn	Phe	Lys	Asn	Ala	Phe	Glu	Ile	Gly	Gly	Ala	Ala	Ile	Leu	645	650	655
Met	Glu	Phe	Ile	Pro	Glu	Leu	Ile	Val	Pro	Ile	Val	Gly	Phe	Phe	Thr	660	665	670
Leu	Glu	Ser	Tyr	Val	Gly	Asn	Lys	Gly	His	Ile	Ile	Met	Thr	Ile	Ser	675	680	685
Asn	Ala	Leu	Lys	Lys	Arg	Asp	Gln	Lys	Trp	Thr	Asp	Met	Tyr	Gly	Leu	690	695	700
Ile	Val	Ser	Gln	Trp	Leu	Ser	Thr	Val	Asn	Thr	Gln	Phe	Tyr	Thr	Ile	705	710	715
Lys	Glu	Arg	Met	Tyr	Asn	Ala	Leu	Asn	Asn	Gln	Ser	Gln	Ala	Ile	Glu	725	730	735

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Lys Ile Ile Glu Asp Gln Tyr Asn Arg Tyr Ser Glu Glu Asp Lys Met
 740 745 750
 Asn Ile Asn Ile Asp Phe Asn Asp Ile Asp Phe Lys Leu Asn Gln Ser
 755 760 765
 Ile Asn Leu Ala Ile Asn Asn Ile Asp Asp Phe Ile Asn Gln Cys Ser
 770 775 780
 Ile Ser Tyr Leu Met Asn Arg Met Ile Pro Leu Ala Val Lys Lys Leu
 785 790 795 800
 Lys Asp Phe Asp Asp Asn Leu Lys Arg Asp Leu Leu Glu Tyr Ile Asp
 805 810 815
 Thr Asn Glu Leu Tyr Leu Leu Asp Glu Val Asn Ile Leu Lys Ser Lys
 820 825 830
 Val Asn Arg His Leu Lys Asp Ser Ile Pro Phe Asp Leu Ser Leu Tyr
 835 840 845
 Thr Lys Asp Thr Ile Leu Ile Gln Val Phe Asn Asn Tyr Ile Ser Asn
 850 855 860
 Ile Ser Ser Asn Ala Ile Leu Ser Leu Ser Tyr Arg Gly Gly Arg Leu
 865 870 875 880
 Ile Asp Ser Ser Gly Tyr Gly Ala Thr Met Asn Val Gly Ser Asp Val
 885 890 895
 Ile Phe Asn Asp Ile Gly Asn Gly Gln Phe Lys Leu Asn Asn Ser Glu
 900 905 910
 Asn Ser Asn Ile Thr Ala His Gln Ser Lys Phe Val Val Tyr Asp Ser
 915 920 925
 Met Phe Asp Asn Phe Ser Ile Asn Phe Trp Val Arg Thr Pro Lys Tyr
 930 935 940
 Asn Asn Asn Asp Ile Gln Thr Tyr Leu Gln Asn Glu Tyr Thr Ile Ile
 945 950 955 960
 Ser Cys Ile Lys Asn Asp Ser Gly Trp Lys Val Ser Ile Lys Gly Asn
 965 970 975
 Arg Ile Ile Trp Thr Leu Ile Asp Val Asn Ala Lys Ser Lys Ser Ile
 980 985 990
 Phe Phe Glu Tyr Ser Ile Lys Asp Asn Ile Ser Asp Tyr Ile Asn Lys
 995 1000 1005
 Trp Phe Ser Ile Thr Ile Thr Asn Asp Arg Leu Gly Asn Ala Asn Ile
 1010 1015 1020
 Tyr Ile Asn Gly Ser Leu Lys Lys Ser Glu Lys Ile Leu Asn Leu Asp
 1025 1030 1035 1040
 Arg Ile Asn Ser Ser Asn Asp Ile Asp Phe Lys Leu Ile Asn Cys Thr
 1045 1050 1055
 Asp Thr Thr Lys Phe Val Trp Ile Lys Asp Phe Asn Ile Phe Gly Arg
 1060 1065 1070
 Glu Leu Asn Ala Thr Glu Val Ser Ser Leu Tyr Trp Ile Gln Ser Ser
 1075 1080 1085
 Thr Asn Thr Leu Lys Asp Phe Trp Gly Asn Pro Leu Arg Tyr Asp Thr
 1090 1095 1100
 Gln Tyr Tyr Leu Phe Asn Gln Gly Met Gln Asn Ile Tyr Ile Lys Tyr
 1105 1110 1115 1120
 Phe Ser Lys Ala Ser Met Gly Glu Thr Ala Pro Arg Thr Asn Phe Asn
 1125 1130 1135
 Asn Ala Ala Ile Asn Tyr Gln Asn Leu Tyr Leu Gly Leu Arg Phe Ile
 1140 1145 1150
 Ile Lys Lys Ala Ser Asn Ser Arg Asn Ile Asn Asn Asp Asn Ile Val
 1155 1160 1165
 Arg Glu Gly Asp Tyr Ile Tyr Leu Asn Ile Asp Asn Ile Ser Asp Glu
 1170 1175 1180
 Ser Tyr Arg Val Tyr Val Leu Val Asn Ser Lys Glu Ile Gln Thr Gln
 1185 1190 1195 1200

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Leu Phe Leu Ala Pro Ile Asn Asp Asp Pro Thr Phe Tyr Asp Val Leu
      1205      1210      1215
Gln Ile Lys Lys Tyr Tyr Glu Lys Thr Thr Tyr Asn Cys Gln Ile Leu
      1220      1225      1230
Cys Glu Lys Asp Thr Lys Thr Phe Gly Leu Phe Gly Ile Gly Lys Phe
      1235      1240      1245
Val Lys Asp Tyr Gly Tyr Val Trp Asp Thr Tyr Asp Asn Tyr Phe Cys
      1250      1255      1260
Ile Ser Gln Trp Tyr Leu Arg Arg Ile Ser Glu Asn Ile Asn Lys Leu
1265      1270      1275      1280
Arg Leu Gly Cys Asn Trp Gln Phe Ile Pro Val Asp Glu Gly Trp Thr
      1285      1290      1295
Glu

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<210> 8

<211> 1315

<212> PRT

<213> Clostridium tetani

<220>

<221> DOMAIN

<222> (1)...(441)

<223> Light chain comprising the enzymatic domain.

<221> DOMAIN

<222> (442)...(870)

<223> Amino-terminal half of heavy chain comprising the translocation domain.

<221> DOMAIN

<222> (871)...(1315)

<223> Carboxyl-terminal half of heavy chain comprising the binding domain.

<400> 8

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Met Pro Ile Thr Ile Asn Asn Phe Arg Tyr Ser Asp Pro Val Asn Asn
 1      5      10      15
Asp Thr Ile Ile Met Met Glu Pro Pro Tyr Cys Lys Gly Leu Asp Ile
      20      25      30
Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Val Pro Glu
      35      40      45
Arg Tyr Glu Phe Gly Thr Lys Pro Glu Asp Phe Asn Pro Pro Ser Ser
      50      55      60
Leu Ile Glu Gly Ala Ser Glu Tyr Tyr Asp Pro Asn Tyr Leu Arg Thr
65      70      75      80
Asp Ser Asp Lys Asp Arg Phe Leu Gln Thr Met Val Lys Leu Phe Asn
      85      90      95
Arg Ile Lys Asn Asn Val Ala Gly Glu Ala Leu Leu Asp Lys Ile Ile
      100      105      110
Asn Ala Ile Pro Tyr Leu Gly Asn Ser Tyr Ser Leu Leu Asp Lys Phe
      115      120      125
Asp Thr Asn Ser Asn Ser Val Ser Phe Asn Leu Leu Glu Gln Asp Pro
      130      135      140
Ser Gly Ala Thr Thr Lys Ser Ala Met Leu Thr Asn Leu Ile Ile Phe
145      150      155      160
Gly Pro Gly Pro Val Leu Asn Lys Asn Glu Val Arg Gly Ile Val Leu
      165      170      175

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Arg	Val	Asp	Asn	Lys	Asn	Tyr	Phe	Pro	Cys	Arg	Asp	Gly	Phe	Gly	Ser
			180					185					190		
Ile	Met	Gln	Met	Ala	Phe	Cys	Pro	Glu	Tyr	Val	Pro	Thr	Phe	Asp	Asn
		195					200					205			
Val	Ile	Glu	Asn	Ile	Thr	Ser	Leu	Thr	Ile	Gly	Lys	Ser	Lys	Tyr	Phe
		210				215					220				
Gln	Asp	Pro	Ala	Leu	Leu	Leu	Met	His	Glu	Leu	Ile	His	Val	Leu	His
225					230					235					240
Gly	Leu	Tyr	Gly	Met	Gln	Val	Ser	Ser	His	Glu	Ile	Ile	Pro	Ser	Lys
			245						250					255	
Gln	Glu	Ile	Tyr	Met	Gln	His	Thr	Tyr	Pro	Ile	Ser	Ala	Glu	Glu	Leu
			260					265					270		
Phe	Thr	Phe	Gly	Gly	Gln	Asp	Ala	Asn	Leu	Ile	Ser	Ile	Asp	Ile	Lys
		275					280					285			
Asn	Asp	Leu	Tyr	Glu	Lys	Thr	Leu	Asn	Asp	Tyr	Lys	Ala	Ile	Ala	Asn
		290				295					300				
Lys	Leu	Ser	Gln	Val	Thr	Ser	Cys	Asn	Asp	Pro	Asn	Ile	Asp	Ile	Asp
305					310					315					320
Ser	Tyr	Lys	Gln	Ile	Tyr	Gln	Gln	Lys	Tyr	Gln	Phe	Asp	Lys	Asp	Ser
			325					330						335	
Asn	Gly	Gln	Tyr	Ile	Val	Asn	Glu	Asp	Lys	Phe	Gln	Ile	Leu	Tyr	Asn
			340					345					350		
Ser	Ile	Met	Tyr	Gly	Phe	Thr	Glu	Ile	Glu	Leu	Gly	Lys	Lys	Phe	Asn
		355					360					365			
Ile	Lys	Thr	Arg	Leu	Ser	Tyr	Phe	Ser	Met	Asn	His	Asp	Pro	Val	Lys
		370				375					380				
Ile	Pro	Asn	Leu	Leu	Asp	Asp	Thr	Ile	Tyr	Asn	Asp	Thr	Glu	Gly	Phe
385					390					395					400
Asn	Ile	Glu	Ser	Lys	Asp	Leu	Lys	Ser	Glu	Tyr	Lys	Gly	Gln	Asn	Met
			405						410					415	
Arg	Val	Asn	Thr	Asn	Ala	Phe	Arg	Asn	Val	Asp	Gly	Ser	Gly	Leu	Val
			420					425					430		
Ser	Lys	Leu	Ile	Gly	Leu	Cys	Lys	Lys	Ile	Ile	Pro	Pro	Thr	Asn	Ile
		435					440					445			
Arg	Glu	Asn	Leu	Tyr	Asn	Arg	Thr	Ala	Ser	Leu	Thr	Asp	Leu	Gly	Gly
		450				455					460				
Glu	Leu	Cys	Ile	Lys	Ile	Lys	Asn	Glu	Asp	Leu	Thr	Phe	Ile	Ala	Glu
465					470				475						480
Lys	Asn	Ser	Phe	Ser	Glu	Glu	Pro	Phe	Gln	Asp	Glu	Ile	Val	Ser	Tyr
			485						490					495	
Asn	Thr	Lys	Asn	Lys	Pro	Leu	Asn	Phe	Asn	Tyr	Ser	Leu	Asp	Lys	Ile
			500					505					510		
Ile	Val	Asp	Tyr	Asn	Leu	Gln	Ser	Lys	Ile	Thr	Leu	Pro	Asn	Asp	Arg
		515					520					525			
Thr	Thr	Pro	Val	Thr	Lys	Gly	Ile	Pro	Tyr	Ala	Pro	Glu	Tyr	Lys	Ser
		530				535					540				
Asn	Ala	Ala	Ser	Thr	Ile	Glu	Ile	His	Asn	Ile	Asp	Asp	Asn	Thr	Ile
545					550					555					560
Tyr	Gln	Tyr	Leu	Tyr	Ala	Gln	Lys	Ser	Pro	Thr	Thr	Leu	Gln	Arg	Ile
			565						570					575	
Thr	Met	Thr	Asn	Ser	Val	Asp	Asp	Ala	Leu	Ile	Asn	Ser	Thr	Lys	Ile
			580					585					590		
Tyr	Ser	Tyr	Phe	Pro	Ser	Val	Ile	Ser	Lys	Val	Asn	Gln	Gly	Ala	Gln
		595					600					605			
Gly	Ile	Leu	Phe	Leu	Gln	Trp	Val	Arg	Asp	Ile	Ile	Asp	Asp	Phe	Thr
					615						620				
Asn	Glu	Ser	Ser	Gln	Lys	Thr	Thr	Ile	Asp	Lys	Ile	Ser	Asp	Val	Ser
625					630					635					640
Thr	Ile	Val	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Val	Lys	Gln	Gly

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				645					650				655				
Tyr	Glu	Gly	Asn	Phe	Ile	Gly	Ala	Leu	Glu	Thr	Thr	Gly	Val	Val	Leu		
			660						665				670				
Leu	Leu	Glu	Tyr	Ile	Pro	Glu	Ile	Thr	Leu	Pro	Val	Ile	Ala	Ala	Leu		
		675					680					685					
Ser	Ile	Ala	Glu	Ser	Ser	Thr	Gln	Lys	Glu	Lys	Ile	Ile	Lys	Thr	Ile		
	690					695					700						
Asp	Asn	Phe	Leu	Glu	Lys	Arg	Tyr	Glu	Lys	Trp	Ile	Glu	Val	Tyr	Lys		
705					710					715					720		
Leu	Val	Lys	Ala	Lys	Trp	Leu	Gly	Thr	Val	Asn	Thr	Gln	Phe	Gln	Lys		
			725						730					735			
Arg	Ser	Tyr	Gln	Met	Tyr	Arg	Ser	Leu	Glu	Tyr	Gln	Val	Asp	Ala	Ile		
			740					745					750				
Lys	Lys	Ile	Asp	Tyr	Glu	Tyr	Lys	Ile	Tyr	Ser	Gly	Pro	Asp	Lys			
	755					760					765						
Glu	Gln	Ile	Ala	Asp	Glu	Ile	Asn	Asn	Leu	Lys	Asn	Lys	Leu	Glu	Glu		
	770					775					780						
Lys	Ala	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Ile	Phe	Met	Arg	Glu	Ser		
785					790					795					800		
Ser	Arg	Ser	Phe	Leu	Val	Asn	Gln	Met	Ile	Asn	Glu	Ala	Lys	Lys	Gln		
			805						810					815			
Leu	Leu	Glu	Phe	Asp	Thr	Gln	Ser	Lys	Asn	Ile	Leu	Met	Gln	Tyr	Ile		
			820					825					830				
Lys	Ala	Asn	Ser	Lys	Phe	Ile	Gly	Ile	Thr	Glu	Leu	Lys	Lys	Leu	Glu		
	835					840					845						
Ser	Lys	Ile	Asn	Lys	Val	Phe	Ser	Thr	Pro	Ile	Pro	Phe	Ser	Tyr	Ser		
	850					855					860						
Lys	Asn	Leu	Asp	Cys	Trp	Val	Asp	Asn	Glu	Glu	Asp	Ile	Asp	Val	Ile		
865					870					875					880		
Leu	Lys	Lys	Ser	Thr	Ile	Leu	Asn	Leu	Asp	Ile	Asn	Asn	Asp	Ile	Ile		
			885						890					895			
Ser	Asp	Ile	Ser	Gly	Phe	Asn	Ser	Ser	Val	Ile	Thr	Tyr	Pro	Asp	Ala		
			900					905					910				
Gln	Leu	Val	Pro	Gly	Ile	Asn	Gly	Lys	Ala	Ile	His	Leu	Val	Asn	Asn		
	915						920					925					
Glu	Ser	Ser	Glu	Val	Ile	Val	His	Lys	Ala	Met	Asp	Ile	Glu	Tyr	Asn		
	930					935					940						
Asp	Met	Phe	Asn	Asn	Phe	Thr	Val	Ser	Phe	Trp	Leu	Arg	Val	Pro	Lys		
945					950					955					960		
Val	Ser	Ala	Ser	His	Leu	Glu	Gln	Tyr	Gly	Thr	Asn	Glu	Tyr	Ser	Ile		
			965						970					975			
Ile	Ser	Ser	Met	Lys	Lys	His	Ser	Leu	Ser	Ile	Gly	Ser	Gly	Trp	Ser		
			980					985					990				
Val	Ser	Leu	Lys	Gly	Asn	Asn	Leu	Ile	Trp	Thr	Leu	Lys	Asp	Ser	Ala		
			995				1000					1005					
Gly	Glu	Val	Arg	Gln	Ile	Thr	Phe	Arg	Asp	Leu	Pro	Asp	Lys	Phe	Asn		
	1010					1015					1020						
Ala	Tyr	Leu	Ala	Asn	Lys	Trp	Val	Phe	Ile	Thr	Ile	Thr	Asn	Asp	Arg		
1025					1030					1035					1040		
Leu	Ser	Ser	Ala	Asn	Leu	Tyr	Ile	Asn	Gly	Val	Leu	Met	Gly	Ser	Ala		
			1045						1050					1055			
Glu	Ile	Thr	Gly	Leu	Gly	Ala	Ile	Arg	Glu	Asp	Asn	Asn	Ile	Thr	Leu		
			1060				1065						1070				
Lys	Leu	Asp	Arg	Cys	Asn	Asn	Asn	Asn	Gln	Tyr	Val	Ser	Ile	Asp	Lys		
			1075				1080						1085				
Phe	Arg	Ile	Phe	Cys	Lys	Ala	Leu	Asn	Pro	Lys	Glu	Ile	Glu	Lys	Leu		
	1090					1095					1100						
Tyr	Thr	Ser	Tyr	Leu	Ser	Ile	Thr	Phe	Leu	Arg	Asp	Phe	Trp	Gly	Asn		

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1105 1110 1115 1120
 Pro Leu Arg Tyr Asp Thr Glu Tyr Tyr Leu Ile Pro Val Ala Ser Ser
 1125 1130 1135
 Ser Lys Asp Val Gln Leu Lys Asn Ile Thr Asp Tyr Met Tyr Leu Thr
 1140 1145 1150
 Asn Ala Pro Ser Tyr Thr Asn Gly Lys Leu Asn Ile Tyr Tyr Arg Arg
 1155 1160 1165
 Leu Tyr Asn Gly Leu Lys Phe Ile Ile Lys Arg Tyr Thr Pro Asn Asn
 1170 1175 1180
 Glu Ile Asp Ser Phe Val Lys Ser Gly Asp Phe Ile Lys Leu Tyr Val
 1185 1190 1195 1200
 Ser Tyr Asn Asn Asn Glu His Ile Val Gly Tyr Pro Lys Asp Gly Asn
 1205 1210 1215
 Ala Phe Asn Asn Leu Asp Arg Ile Leu Arg Val Gly Tyr Asn Ala Pro
 1220 1225 1230
 Gly Ile Pro Leu Tyr Lys Lys Met Glu Ala Val Lys Leu Arg Asp Leu
 1235 1240 1245
 Lys Thr Tyr Ser Val Gln Leu Lys Leu Tyr Asp Asp Lys Asn Ala Ser
 1250 1255 1260
 Leu Gly Leu Val Gly Thr His Asn Gly Gln Ile Gly Asn Asp Pro Asn
 1265 1270 1275 1280
 Arg Asp Ile Leu Ile Ala Ser Asn Trp Tyr Phe Asn His Leu Lys Asp
 1285 1290 1295
 Lys Ile Leu Gly Cys Asp Trp Tyr Phe Val Pro Thr Asp Glu Gly Trp
 1300 1305 1310
 Thr Asn Asp
 1315

<210> 9
 <211> 425
 <212> PRT
 <213> Homo sapiens

<220>
 <221> PEPTIDE
 <222> (1)...(425)
 <223> PAR1

<400> 9
 Met Gly Pro Arg Arg Leu Leu Leu Val Ala Ala Cys Phe Ser Leu Cys
 1 5 10 15
 Gly Pro Leu Leu Ser Ala Arg Thr Arg Ala Arg Arg Pro Glu Ser Lys
 20 25 30
 Ala Thr Asn Ala Thr Leu Asp Pro Arg Ser Phe Leu Leu Arg Asn Pro
 35 40 45
 Asn Asp Lys Tyr Glu Pro Phe Trp Glu Asp Glu Glu Lys Asn Glu Ser
 50 55 60
 Gly Leu Thr Glu Tyr Arg Leu Val Ser Ile Asn Lys Ser Ser Pro Leu
 65 70 75 80
 Gln Lys Gln Leu Pro Ala Phe Ile Ser Glu Asp Ala Ser Gly Tyr Leu
 85 90 95
 Thr Ser Ser Trp Leu Thr Leu Phe Val Pro Ser Val Tyr Thr Gly Val
 100 105 110
 Phe Val Val Ser Leu Pro Leu Asn Ile Met Ala Ile Val Val Phe Ile
 115 120 125
 Leu Lys Met Lys Val Lys Lys Pro Ala Val Val Tyr Met Leu His Leu
 130 135 140
 Ala Thr Ala Asp Val Leu Phe Val Ser Val Leu Pro Phe Lys Ile Ser

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145          150          155          160
Tyr Tyr Phe Ser Gly Ser Asp Trp Gln Phe Gly Ser Glu Leu Cys Arg
          165          170          175
Phe Val Thr Ala Ala Phe Tyr Cys Asn Met Tyr Ala Ser Ile Leu Leu
          180          185          190
Met Thr Val Ile Ser Ile Asp Arg Phe Leu Ala Val Val Tyr Pro Met
          195          200          205
Gln Ser Leu Ser Trp Arg Thr Leu Gly Arg Ala Ser Phe Thr Cys Leu
          210          215          220
Ala Ile Trp Ala Leu Ala Ile Ala Gly Val Val Pro Leu Leu Leu Lys
225          230          235          240
Glu Gln Thr Ile Gln Val Pro Gly Leu Asn Ile Thr Thr Cys His Asp
          245          250          255
Val Leu Asn Glu Thr Leu Leu Glu Gly Tyr Tyr Ala Tyr Tyr Phe Ser
          260          265          270
Ala Phe Ser Ala Val Phe Phe Phe Val Pro Leu Ile Ile Ser Thr Val
          275          280          285
Cys Tyr Val Ser Ile Ile Arg Cys Leu Ser Ser Ser Ala Val Ala Asn
          290          295          300
Arg Ser Lys Lys Ser Arg Ala Leu Phe Leu Ser Ala Ala Val Phe Cys
305          310          315          320
Ile Phe Ile Ile Cys Phe Gly Pro Thr Asn Val Leu Leu Ile Ala His
          325          330          335
Tyr Ser Phe Leu Ser His Thr Ser Thr Thr Glu Ala Ala Tyr Phe Ala
          340          345          350
Tyr Leu Leu Cys Val Cys Val Ser Ser Ile Ser Cys Cys Ile Asp Pro
          355          360          365
Leu Ile Tyr Tyr Tyr Ala Ser Ser Glu Cys Gln Arg Tyr Val Tyr Ser
          370          375          380
Ile Leu Cys Cys Lys Glu Ser Ser Asp Pro Ser Ser Tyr Asn Ser Ser
385          390          395          400
Gly Gln Leu Met Ala Ser Lys Met Asp Thr Cys Ser Ser Asn Leu Asn
          405          410          415
Asn Ser Ile Tyr Lys Lys Leu Leu Thr
          420          425

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<210> 10

<211> 397

<212> PRT

<213> Homo sapiens

<220>

<221> PEPTIDE

<222> (1)...(397)

<223> PAR2

<400> 10

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Met Arg Ser Pro Ser Ala Ala Trp Leu Leu Gly Ala Ala Ile Leu Leu
 1          5          10          15
Ala Ala Ser Leu Ser Cys Ser Gly Thr Ile Gln Gly Thr Asn Arg Ser
          20          25          30
Ser Lys Gly Arg Ser Leu Ile Gly Lys Val Asp Gly Thr Ser His Val
          35          40          45
Thr Gly Lys Gly Val Thr Val Glu Thr Val Phe Ser Val Asp Glu Phe
          50          55          60
Ser Ala Ser Val Leu Thr Gly Lys Leu Thr Thr Val Phe Leu Pro Ile
65          70          75          80
Val Tyr Thr Ile Val Phe Val Val Gly Leu Pro Ser Asn Gly Met Ala

```

Li *et al.*, Degradable Clostridial Toxins

```

      85      90      95
Leu Trp Val Phe Leu Phe Arg Thr Lys Lys Lys His Pro Ala Val Ile
      100      105      110
Tyr Met Ala Asn Leu Ala Leu Ala Asp Leu Leu Ser Val Ile Trp Phe
      115      120      125
Pro Leu Lys Ile Ala Tyr His Ile His Gly Asn Asn Trp Ile Tyr Gly
      130      135      140
Glu Ala Leu Cys Asn Val Leu Ile Gly Phe Phe Tyr Gly Asn Met Tyr
145      150      155      160
Cys Ser Ile Leu Phe Met Thr Cys Leu Ser Val Gln Arg Tyr Trp Val
      165      170      175
Ile Val Asn Pro Met Gly His Ser Arg Lys Lys Ala Asn Ile Ala Ile
      180      185      190
Gly Ile Ser Leu Ala Ile Trp Leu Leu Ile Leu Leu Val Thr Ile Pro
      195      200      205
Leu Tyr Val Val Lys Gln Thr Ile Phe Ile Pro Ala Leu Asn Ile Thr
210      215      220
Thr Cys His Asp Val Leu Pro Glu Gln Leu Leu Val Gly Asp Met Phe
225      230      235      240
Asn Tyr Phe Leu Ser Leu Ala Ile Gly Val Phe Leu Phe Pro Ala Phe
      245      250      255
Leu Thr Ala Ser Ala Tyr Val Leu Met Ile Arg Met Leu Arg Ser Ser
      260      265      270
Ala Met Asp Glu Asn Ser Glu Lys Lys Arg Lys Arg Ala Ile Lys Leu
275      280      285
Ile Val Thr Val Leu Ala Met Tyr Leu Ile Cys Phe Thr Pro Ser Asn
290      295      300
Leu Leu Leu Val Val His Tyr Phe Leu Ile Lys Ser Gln Gly Gln Ser
305      310      315      320
His Val Tyr Ala Leu Tyr Ile Val Ala Leu Cys Leu Ser Thr Leu Asn
      325      330      335
Ser Cys Ile Asp Pro Phe Val Tyr Tyr Phe Val Ser His Asp Phe Arg
      340      345      350
Asp His Ala Lys Asn Ala Leu Leu Cys Arg Ser Val Arg Thr Val Lys
      355      360      365
Gln Met Gln Val Ser Leu Thr Ser Lys Lys His Ser Arg Lys Ser Ser
      370      375      380
Ser Tyr Ser Ser Ser Ser Thr Thr Val Lys Thr Ser Tyr
385      390      395

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<210> 11

<211> 374

<212> PRT

<213> Homo sapiens

<220>

<221> PEPTIDE

<222> (1)...(374)

<223> PAR3

<400> 11

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Met Lys Ala Leu Ile Phe Ala Ala Ala Gly Leu Leu Leu Leu Leu Pro
 1      5      10      15
Thr Phe Cys Gln Ser Gly Met Glu Asn Asp Thr Asn Asn Leu Ala Lys
      20      25      30
Pro Thr Leu Pro Ile Lys Thr Phe Arg Gly Ala Pro Pro Asn Ser Phe
      35      40      45
Glu Glu Phe Pro Phe Ser Ala Leu Glu Gly Trp Thr Gly Ala Thr Ile

```


Li *et al.*, Degradable Clostridial Toxins

50		55		60											
Thr	Val	Lys	Ile	Lys	Cys	Pro	Glu	Glu	Ser	Ala	Ser	His	Leu	His	Val
65					70					75					80
Lys	Asn	Ala	Thr	Met	Gly	Tyr	Leu	Thr	Ser	Ser	Leu	Ser	Thr	Lys	Leu
				85					90					95	
Ile	Pro	Ala	Ile	Tyr	Leu	Leu	Val	Phe	Val	Val	Gly	Val	Pro	Ala	Asn
			100					105					110		
Ala	Val	Thr	Leu	Trp	Met	Leu	Phe	Phe	Arg	Thr	Arg	Ser	Ile	Cys	Thr
		115					120					125			
Thr	Val	Phe	Tyr	Thr	Asn	Leu	Ala	Ile	Ala	Asp	Phe	Leu	Phe	Cys	Val
130					135						140				
Thr	Leu	Pro	Phe	Lys	Ile	Ala	Tyr	His	Leu	Asn	Gly	Asn	Asn	Trp	Val
145					150					155					160
Phe	Gly	Glu	Val	Leu	Cys	Arg	Ala	Thr	Thr	Val	Ile	Phe	Tyr	Gly	Asn
			165					170						175	
Met	Tyr	Cys	Ser	Ile	Leu	Leu	Leu	Ala	Cys	Ile	Ser	Ile	Asn	Arg	Tyr
		180					185						190		
Leu	Ala	Ile	Val	His	Pro	Phe	Thr	Tyr	Arg	Gly	Leu	Pro	Lys	His	Thr
	195						200					205			
Tyr	Ala	Leu	Val	Thr	Cys	Gly	Leu	Val	Trp	Ala	Thr	Val	Phe	Leu	Tyr
210					215					220					
Met	Leu	Pro	Phe	Phe	Ile	Leu	Lys	Gln	Glu	Tyr	Tyr	Leu	Val	Gln	Pro
225				230					235						240
Asp	Ile	Thr	Thr	Cys	His	Asp	Val	His	Asn	Thr	Cys	Glu	Ser	Ser	Ser
			245					250						255	
Pro	Phe	Gln	Leu	Tyr	Tyr	Phe	Ile	Ser	Leu	Ala	Phe	Phe	Gly	Phe	Leu
		260					265						270		
Ile	Pro	Phe	Val	Leu	Ile	Ile	Tyr	Cys	Tyr	Ala	Ala	Ile	Ile	Arg	Thr
	275						280					285			
Leu	Asn	Ala	Tyr	Asp	His	Arg	Trp	Leu	Trp	Tyr	Val	Lys	Ala	Ser	Leu
290					295						300				
Leu	Ile	Leu	Val	Ile	Phe	Thr	Ile	Cys	Phe	Ala	Pro	Ser	Asn	Ile	Ile
305				310					315						320
Leu	Ile	Ile	His	His	Ala	Asn	Tyr	Tyr	Tyr	Asn	Asn	Thr	Asp	Gly	Leu
			325					330						335	
Tyr	Phe	Ile	Tyr	Leu	Ile	Ala	Leu	Cys	Leu	Gly	Ser	Leu	Asn	Ser	Cys
		340					345						350		
Leu	Asp	Pro	Phe	Leu	Tyr	Phe	Leu	Met	Ser	Lys	Thr	Arg	Asn	His	Ser
	355						360					365			
Thr	Ala	Tyr	Leu	Thr	Lys										
370															

<210> 12

<211> 385

<212> PRT

<213> Homo sapiens

<220>

<221> PEPTIDE

<222> (1)...(385)

<223> PAR4

<400> 12

Met	Trp	Gly	Arg	Leu	Leu	Leu	Trp	Pro	Leu	Val	Leu	Gly	Phe	Ser	Leu
1				5					10					15	
Ser	Gly	Gly	Thr	Gln	Thr	Pro	Ser	Val	Tyr	Asp	Glu	Ser	Gly	Ser	Thr
			20				25						30		
Gly	Gly	Gly	Asp	Asp	Ser	Thr	Pro	Ser	Ile	Leu	Pro	Ala	Pro	Arg	Gly

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	35		40		45														
Tyr	Pro	Gly	Gln	Val	Cys	Ala	Asn	Asp	Ser	Asp	Thr	Leu	Glu	Leu	Pro				
	50					55					60								
Asp	Ser	Ser	Arg	Ala	Leu	Leu	Leu	Gly	Trp	Val	Pro	Thr	Arg	Leu	Val				
65					70					75					80				
Pro	Ala	Leu	Tyr	Gly	Leu	Val	Leu	Val	Val	Gly	Leu	Pro	Ala	Asn	Gly				
				85					90					95					
Leu	Ala	Leu	Trp	Val	Leu	Ala	Thr	Gln	Ala	Pro	Arg	Leu	Pro	Ser	Thr				
			100					105					110						
Met	Leu	Leu	Met	Asn	Leu	Ala	Thr	Ala	Asp	Leu	Leu	Leu	Ala	Leu	Ala				
	115						120					125							
Leu	Pro	Pro	Arg	Ile	Ala	Tyr	His	Leu	Arg	Gly	Gln	Arg	Trp	Pro	Phe				
	130					135					140								
Gly	Glu	Ala	Ala	Cys	Arg	Leu	Ala	Thr	Ala	Ala	Leu	Tyr	Gly	His	Met				
145				150					155					160					
Tyr	Gly	Ser	Val	Leu	Leu	Leu	Ala	Ala	Val	Ser	Leu	Asp	Arg	Tyr	Leu				
			165					170					175						
Ala	Leu	Val	His	Pro	Leu	Arg	Ala	Arg	Ala	Leu	Arg	Gly	Arg	Arg	Leu				
		180					185					190							
Ala	Leu	Gly	Leu	Cys	Met	Ala	Ala	Trp	Leu	Met	Ala	Ala	Ala	Leu	Ala				
	195					200					205								
Leu	Pro	Leu	Thr	Leu	Gln	Arg	Gln	Thr	Phe	Arg	Leu	Ala	Arg	Ser	Asp				
	210				215						220								
Arg	Val	Leu	Cys	His	Asp	Ala	Leu	Pro	Leu	Asp	Ala	Gln	Ala	Ser	His				
225				230					235					240					
Trp	Gln	Pro	Ala	Phe	Thr	Cys	Leu	Ala	Leu	Gly	Cys	Phe	Leu	Pro					
			245					250				255							
Leu	Leu	Ala	Met	Leu	Leu	Cys	Tyr	Gly	Ala	Thr	Leu	His	Thr	Leu	Ala				
		260					265					270							
Ala	Ser	Gly	Arg	Arg	Tyr	Gly	His	Ala	Leu	Arg	Leu	Thr	Ala	Val	Val				
		275				280					285								
Leu	Ala	Ser	Ala	Val	Ala	Phe	Phe	Val	Pro	Ser	Asn	Leu	Leu	Leu	Leu				
	290				295						300								
Leu	His	Tyr	Ser	Asp	Pro	Ser	Pro	Ser	Ala	Trp	Gly	Asn	Leu	Tyr	Gly				
305				310					315					320					
Ala	Tyr	Val	Pro	Ser	Leu	Ala	Leu	Ser	Thr	Leu	Asn	Ser	Cys	Val	Asp				
			325					330					335						
Pro	Phe	Ile	Tyr	Tyr	Tyr	Val	Ser	Ala	Glu	Phe	Arg	Asp	Lys	Val	Arg				
		340				345					350								
Ala	Gly	Leu	Phe	Gln	Arg	Ser	Pro	Gly	Asp	Thr	Val	Ala	Ser	Lys	Ala				
	355					360					365								
Ser	Ala	Glu	Gly	Gly	Ser	Arg	Gly	Met	Gly	Thr	His	Ser	Ser	Leu	Leu				
	370				375					380									

Gln
385

<210> 13

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Hexapeptide comprising the tethered ligand of PAR1

<400> 13

Ser Phe Phe Leu Arg Asn

Li *et al.*, Degradable Clostridial Toxins

1

5

<210> 14

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered
ligand of PAR1

<400> 14

Ser Phe Phe Leu Arg Asn

1

5

<210> 15

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered
ligand of PAR1

<400> 15

Thr Phe Leu Leu Arg Asn

1

5

<210> 16

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered
ligand of PAR1

<400> 16

Gly Phe Pro Gly Lys Phe

1

5

<210> 17

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

Li *et al.*, Degradable Clostridial Toxins

<223> Variant of hexapeptide comprising the tethered
ligand of PAR1

<400> 17

Gly Tyr Pro Ala Lys Phe
1 5

<210> 18

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered
ligand of PAR1

<400> 18

Gly Tyr Pro Leu Lys Phe
1 5

<210> 19

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered
ligand of PAR1

<400> 19

Gly Tyr Pro Ile Lys Phe
1 5

<210> 20

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered
ligand of PAR1

<221> MOD_RES

<222> (2)...(2)

<223> Xaa is parafluoro-phenylalanine (F).

<400> 20

Gly Xaa Pro Gly Lys Phe
1 5

Li *et al.*, Degradable Clostridial Toxins

<210> 21
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR1

<221> MOD_RES
<222> (4)...(4)
<223> Xaa is cyclohexylalanine (Cha).

<400> 21
Gly Tyr Pro Xaa Lys Phe
1 5

<210> 22
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR1

<221> MOD_RES
<222> (2)...(2)
<223> Xaa is parafluoro-phenylalanine (F).

<221> MOD_RES
<222> (3)...(3)
<223> Xaa is cyclohexylalanine (Cha).

<221> MOD_RES
<222> (4)...(4)
<223> Xaa is cyclohexylalanine (Cha).

<400> 22
Ser Xaa Xaa Xaa Arg Lys
1 5

<210> 23
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)

Li *et al.*, Degradable Clostridial Toxins

<223> Variant of hexapeptide comprising the tethered ligand of PAR1

<221> MOD_RES

<222> (2)...(2)

<223> Xaa is parafluoro-phenylalanine (F).

<221> MOD_RES

<222> (3)...(3)

<223> Xaa is cyclohexylalanine (Cha).

<221> MOD_RES

<222> (4)...(4)

<223> Xaa is cyclohexylalanine (Cha).

<221> MOD_RES

<222> (5)...(5)

<223> Xaa is homoarginine (homoR).

<400> 23

Ser Xaa Xaa Xaa Xaa Lys

1

5

<210> 24

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Hexapeptide comprising the tethered ligand of PAR2

<400> 24

Ser Leu Ile Gly Lys Val

1

5

<210> 25

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered ligand of PAR2

<400> 25

Ser Leu Ile Gly Arg Leu

1

5

<210> 26

<211> 6

<212> PRT

<213> Artificial Sequence

Li *et al.*, Degradable Clostridial Toxins

<223> Variant of hexapeptide comprising the tethered ligand of PAR1

<221> MOD_RES
<222> (2)...(2)
<223> Xaa is parafluoro-phenylalanine (F).

<221> MOD_RES
<222> (3)...(3)
<223> Xaa is cyclohexylalanine (Cha).

<221> MOD_RES
<222> (4)...(4)
<223> Xaa is cyclohexylalanine (Cha).

<221> MOD_RES
<222> (5)...(5)
<223> Xaa is homoarginine (homoR).

<400> 23
Ser Xaa Xaa Xaa Xaa Lys
1 5

<210> 24
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Hexapeptide comprising the tethered ligand of PAR2

<400> 24
Ser Leu Ile Gly Lys Val
1 5

<210> 25
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered ligand of PAR2

<400> 25
Ser Leu Ile Gly Arg Leu
1 5

<210> 26
<211> 6
<212> PRT
<213> Artificial Sequence

Li *et al.*, Degradable Clostridial Toxins

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Hexapeptide comprising the tethered ligand of PAR3

<400> 26
Thr Phe Arg Gly Ala Pro
1 5

<210> 27
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR3

<400> 27
Ser Phe Asn Gly Gly Pro
1 5

<210> 28
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Hexapeptide comprising the tethered ligand of PAR4

<400> 28
Gly Tyr Pro Gly Gln Val
1 5

<210> 29
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 29
Ala Tyr Pro Gly Lys Phe
1 5

<210> 30

Li *et al.*, Degradable Clostridial Toxins

<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 30
Thr Tyr Pro Gly Lys Phe
1 5

<210> 31
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 31
Gly Tyr Pro Gly Lys Tyr
1 5

<210> 32
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 32
Gly Tyr Pro Gly Lys Trp
1 5

<210> 33
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 33

Li *et al.*, Degradable Clostridial Toxins

Gly Tyr Pro Gly Lys Lys
1 5

<210> 34
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 34
Gly Tyr Pro Gly Lys Phe
1 5

<210> 35
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 35
Gly Tyr Pro Gly Arg Phe
1 5

<210> 36
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 36
Gly Tyr Pro Gly Phe Lys
1 5

<210> 37
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE

Li *et al.*, Degradable Clostridial Toxins

<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 37
Gly Tyr Pro Ala Lys Phe
1 5

<210> 38
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 38
Gly Phe Pro Gly Lys Phe
1 5

<210> 39
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 39
Gly Phe Pro Gly Lys Pro
1 5

<210> 40
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 40
Ser Tyr Pro Gly Lys Phe
1 5

<210> 41
<211> 6

Li *et al.*, Degradable Clostridial Toxins

<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 41
Ser Tyr Pro Ala Lys Phe
1 5

<210> 42
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 42
Ser Tyr Pro Gly Arg Phe
1 5

<210> 43
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<400> 43
Ser Tyr Ala Gly Lys Phe
1 5

<210> 44
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(6)
<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<221> MOD_RES
<222> (5)...(5)

Li *et al.*, Degradable Clostridial Toxins

<223> Xaa is ornithine (Orn).

<400> 44

Gly Tyr Pro Gly Xaa Phe
1 5

<210> 45

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<221> MOD_RES

<222> (2)...(2)

<223> Xaa is parafluoro-phenylalanine (F).

<400> 45

Gly Xaa Pro Gly Lys Phe
1 5

<210> 46

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<221> MOD_RES

<222> (5)...(5)

<223> Xaa is homoarginine (homoR).

<400> 46

Gly Tyr Pro Gly Xaa Phe
1 5

<210> 47

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(6)

<223> Variant of hexapeptide comprising the tethered
ligand of PAR4

<221> MOD_RES

Li *et al.*, Degradable Clostridial Toxins

<222> (5)...(5)

<223> Xaa is homoarginine (homoR).

<400> 47

Ser Tyr Pro Gly Xaa Phe
1 5

<210> 48

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(5)

<223> Flexible peptide spacer

<400> 48

Gly Gly Gly Gly Ser
1 5

<210> 49

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(5)

<223> Flexible peptide spacer

<400> 49

Glu Ala Ala Ala Lys
1 5

<210> 50

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<221> SITE

<222> (1)...(5)

<223> Bovine enterokinase protease cleavage site.

<400> 50

Asp Asp Asp Asp Lys
1 5

<210> 51

<211> 7

<212> PRT

<213> Artificial Sequence

Li et al., Degradable Clostridial Toxins

<220>
<221> SITE
<222> (1)...(7)
<223> Tobacco Etch Virus protease cleavage site.

<400> 51
Glu Asn Leu Tyr Phe Gln Gly
1 5

<210> 52
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(7)
<223> Tobacco Etch Virus protease cleavage site.

<400> 52
Glu Asn Leu Tyr Phe Gln Ser
1 5

<210> 53
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(7)
<223> Tobacco Etch Virus protease cleavage site.

<400> 53
Glu Asn Ile Tyr Thr Gln Gly
1 5

<210> 54
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(7)
<223> Tobacco Etch Virus protease cleavage site.

<400> 54
Glu Asn Ile Tyr Thr Gln Ser
1 5

<210> 55
<211> 7
<212> PRT
<213> Artificial Sequence

Li *et al.*, Degradable Clostridial Toxins

<220>
<221> SITE
<222> (1)...(7)
<223> Tobacco Etch Virus protease cleavage site.

<400> 55
Glu Asn Ile Tyr Leu Gln Gly
1 5

<210> 56
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(7)
<223> Tobacco Etch Virus protease cleavage site.

<400> 56
Glu Asn Ile Tyr Leu Gln Ser
1 5

<210> 57
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(7)
<223> Tobacco Etch Virus protease cleavage site.

<400> 57
Glu Asn Val Tyr Phe Gln Gly
1 5

<210> 58
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(7)
<223> Tobacco Etch Virus protease cleavage site.

<400> 58
Glu Asn Val Tyr Ser Gln Ser
1 5

<210> 59
<211> 7
<212> PRT

Li *et al.*, Degradable Clostridial Toxins

<213> Artificial Sequence

<220>

<221> SITE

<222> (1)...(7)

<223> Tobacco Etch Virus protease cleavage site.

<400> 59

Glu Asn Val Tyr Ser Gln Gly
1 5

<210> 60

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> SITE

<222> (0)...(0)

<223> Tobacco Etch Virus protease cleavage site.

<400> 60

Glu Asn Val Tyr Ser Gln Ser
1 5

<210> 61

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> SITE

<222> (1)...(7)

<223> Human Rhinovirus 3C protease cleavage site.

<400> 61

Glu Ala Leu Phe Gln Gly Pro
1 5

<210> 62

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> SITE

<222> (1)...(7)

<223> Human Rhinovirus 3C protease cleavage site.

<400> 62

Glu Val Leu Phe Gln Gly Pro
1 5

<210> 63

<211> 7

Li et al., Degradable Clostridial Toxins

<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(7)
<223> Human Rhinovirus 3C protease cleavage site.

<400> 63
Glu Leu Leu Phe Gln Gly Pro
1 5

<210> 64
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(7)
<223> Human Rhinovirus 3C protease cleavage site.

<400> 64
Asp Ala Leu Phe Gln Gly Pro
1 5

<210> 65
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(7)
<223> Human Rhinovirus 3C protease cleavage site.

<400> 65
Asp Val Leu Phe Gln Gly Pro
1 5

<210> 66
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (0)...(0)
<223> Human Rhinovirus 3C protease cleavage site.

<400> 66
Asp Leu Leu Phe Gln Gly Pro
1 5

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<210> 67
 <211> 98
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> SITE
 <222> (1)...(98)
 <223> SUMO/ULP-1 protease cleavage site.

<400> 67
 Met Ala Asp Ser Glu Val Asn Gln Glu Ala Lys Pro Glu Val Lys Pro
 1 5 10 15
 Glu Val Lys Pro Glu Thr His Ile Asn Leu Lys Val Ser Asp Gly Ser
 20 25 30
 Ser Glu Ile Phe Phe Lys Ile Lys Lys Thr Thr Pro Leu Arg Arg Leu
 35 40 45
 Met Glu Ala Phe Ala Lys Arg Gln Gly Lys Glu Met Asp Ser Leu Arg
 50 55 60
 Phe Leu Tyr Asp Gly Ile Arg Ile Gln Ala Asp Gln Thr Pro Glu Asp
 65 70 75 80
 Leu Asp Met Glu Asp Asn Asp Ile Ile Glu Ala His Arg Glu Gln Ile
 85 90 95
 Gly Gly

<210> 68
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> SITE
 <222> (1)...(4)
 <223> Thrombin protease cleavage site.

<400> 68
 Gly Val Arg Gly
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<210> 69
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> SITE
 <222> (1)...(4)
 <223> Thrombin protease cleavage site.

<400> 69
 Ser Ala Arg Gly
 1

<210> 70
 <211> 4

Li *et al.*, Degradable Clostridial Toxins

<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(4)
<223> Thrombin protease cleavage site.

<400> 70
Ser Leu Arg Gly
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<210> 71
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(4)
<223> Thrombin protease cleavage site.

<400> 71
Asp Gly Arg Ile
1

<210> 72
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(4)
<223> Thrombin protease cleavage site.

<400> 72
Gln Gly Lys Ile
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<210> 73
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(6)
<223> Thrombin protease cleavage site.

<400> 73
Leu Val Pro Arg Gly Ser
1 5

<210> 74

Li *et al.*, Degradable Clostridial Toxins

<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(6)
<223> Thrombin protease cleavage site.

<400> 74
Leu Val Pro Lys Gly Ser
1 5

<210> 75
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(6)
<223> Thrombin protease cleavage site.

<400> 75
Phe Ile Pro Arg Thr Phe
1 5

<210> 76
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(6)
<223> Thrombin protease cleavage site.

<400> 76
Val Leu Pro Arg Ser Phe
1 5

<210> 77
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(6)
<223> Thrombin protease cleavage site.

<400> 77
Ile Val Pro Arg Ser Phe
1 5

Li *et al.*, Degradable Clostridial Toxins

<210> 78
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(6)
<223> Thrombin protease cleavage site.

<400> 78
Ile Val Pro Arg Gly Tyr
1 5

<210> 79
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(6)
<223> Thrombin protease cleavage site.

<400> 79
Val Val Pro Arg Gly Val
1 5

<210> 80
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(6)
<223> Thrombin protease cleavage site.

<400> 80
Val Leu Pro Arg Leu Ile
1 5

<210> 81
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(6)
<223> Thrombin protease cleavage site.

<400> 81
Val Met Pro Arg Ser Leu
1 5

Li *et al.*, Degradable Clostridial Toxins

<210> 82
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(6)
<223> Thrombin protease cleavage site.

<400> 82
Met Phe Pro Arg Ser Leu
1 5

<210> 83
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(4)
<223> Coagulation Factor Xa protease cleavage site.

<400> 83
Ile Asp Gly Arg
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<210> 84
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)...(4)
<223> Coagulation Factor Xa protease cleavage site.

<400> 84
Ile Glu Gly Arg
1

<210> 85
<211> 1350
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)...(1350)
<223> BoNT/A-ED-PAR1-Thrombin

<400> 85
Met Gly Pro Arg Arg Leu Leu Leu Val Ala Ala Cys Phe Ser Leu Cys
1 5 10 15

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Gly	Pro	Leu	Leu	Ser	Ala	Arg	Thr	Arg	Ala	Arg	Arg	Pro	Glu	Ser	Lys
			20					25					30		
Ala	Thr	Asn	Ala	Thr	Leu	Asp	Pro	Arg	Ser	Phe	Leu	Leu	Arg	Asn	Pro
		35					40					45			
Asn	Asp	Lys	Tyr	Glu	Pro	Phe	Pro	Phe	Val	Asn	Lys	Gln	Phe	Asn	Tyr
	50					55					60				
Lys	Asp	Pro	Val	Asn	Gly	Val	Asp	Ile	Ala	Tyr	Ile	Lys	Ile	Pro	Asn
65					70					75					80
Ala	Gly	Gln	Met	Gln	Pro	Val	Lys	Ala	Phe	Lys	Ile	His	Asn	Lys	Ile
				85					90					95	
Trp	Val	Ile	Pro	Glu	Arg	Asp	Thr	Phe	Thr	Asn	Pro	Glu	Glu	Gly	Asp
			100					105					110		
Leu	Asn	Pro	Pro	Pro	Glu	Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp
		115					120					125			
Ser	Thr	Tyr	Leu	Ser	Thr	Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly
	130					135					140				
Val	Thr	Lys	Leu	Phe	Glu	Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met
145					150					155					160
Leu	Leu	Thr	Ser	Ile	Val	Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr
				165					170					175	
Ile	Asp	Thr	Glu	Leu	Lys	Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile
		180						185					190		
Gln	Pro	Asp	Gly	Ser	Tyr	Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile
		195					200					205			
Gly	Pro	Ser	Ala	Asp	Ile	Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His
	210					215					220				
Glu	Val	Leu	Asn	Leu	Thr	Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile
225					230					235					240
Arg	Phe	Ser	Pro	Asp	Phe	Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val
				245					250					255	
Asp	Thr	Asn	Pro	Leu	Leu	Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala
			260					265					270		
Val	Thr	Leu	Ala	His	Glu	Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly
		275					280					285			
Ile	Ala	Ile	Asn	Pro	Asn	Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr
	290					295					300				
Tyr	Glu	Met	Ser	Gly	Leu	Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe
305					310					315					320
Gly	Gly	His	Asp	Ala	Lys	Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe
				325					330					335	
Arg	Leu	Tyr	Tyr	Asn	Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	
		340					345					350			
Lys	Ala	Lys	Ser	Ile	Val	Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys
		355					360					365			
Asn	Val	Phe	Lys	Glu	Lys	Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys
	370					375					380				
Phe	Ser	Val	Asp	Lys	Leu	Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr
385					390					395					400
Glu	Ile	Tyr	Thr	Glu	Asp	Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn
				405					410					415	
Arg	Lys	Thr	Tyr	Leu	Asn	Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile
		420						425					430		
Val	Pro	Lys	Val	Asn	Tyr	Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn
		435					440					445			
Thr	Asn	Leu	Ala	Ala	Asn	Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn
	450					455					460				
Met	Asn	Phe	Thr	Lys	Leu	Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr
465					470					475					480
Lys	Leu	Leu	Cys	Val	Arg	Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu

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				485				490					495			
Asp	Lys	Gly	Tyr	Asn	Lys	Ala	Leu	Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn	
			500					505					510			
Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	
		515					520					525				
Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	
	530					535					540					
Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	
545				550					555						560	
Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	
			565						570					575		
Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	
			580					585					590			
Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu	
		595					600					605				
Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	
	610					615					620					
Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe	
625					630					635					640	
Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	
			645						650					655		
Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	
		660					665						670			
Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	
	675					680						685				
Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	
	690					695					700					
Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	
705					710					715					720	
Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	
			725						730					735		
Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	
			740					745					750			
Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	
		755					760					765				
Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	
	770					775					780					
Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	
785					790					795					800	
Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	
			805						810					815		
Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	
			820					825					830			
Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	
		835					840					845				
Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	
	850					855					860					
Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	
865					870					875					880	
Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	Asp	Lys	Val	Asn	
			885						890					895		
Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	Lys	Tyr	Val	Asp	
		900						905					910			
Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	
		915					920						925			
Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	
	930					935						940				
Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	
945					950					955					960	

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Asp Pro Ile Asp Lys Asn Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser
      965                      970                      975
Lys Ile Glu Val Ile Leu Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr
      980                      985                      990
Glu Asn Phe Ser Thr Ser Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn
      995                      1000                     1005
Ser Ile Ser Leu Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn
      1010                     1015                     1020
Asn Ser Gly Trp Lys Val Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr
      1025                     1030                     1035                     1040
Leu Gln Asp Thr Gln Glu Ile Lys Gln Arg Val Val Phe Lys Tyr Ser
      1045                     1050                     1055
Gln Met Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr
      1060                     1065                     1070
Ile Thr Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg
      1075                     1080                     1085
Leu Ile Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser
      1090                     1095                     1100
Asn Asn Ile Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr
      1105                     1110                     1115                     1120
Ile Trp Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys
      1125                     1130                     1135
Glu Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys
      1140                     1145                     1150
Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu
      1155                     1160                     1165
Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile
      1170                     1175                     1180
Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr
      1185                     1190                     1195                     1200
Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile
      1205                     1210                     1215
Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp
      1220                     1225                     1230
Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala
      1235                     1240                     1245
Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu
      1250                     1255                     1260
Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val Val Met Lys Ser Lys
      1265                     1270                     1275                     1280
Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn
      1285                     1290                     1295
Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile
      1300                     1305                     1310
Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser
      1315                     1320                     1325
Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly
      1330                     1335                     1340
Trp Gly Glu Arg Pro Leu
      1345                     1350

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<210> 86

<211> 1342

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

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<222> (1)...(1342)

<223> BoNT/A-ED-PAR1-Xa

<400> 86

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Met Gly Pro Arg Arg Leu Leu Leu Val Ala Ala Cys Phe Ser Leu Cys
 1           5           10           15
Gly Pro Leu Leu Ser Ala Arg Thr Arg Ala Arg Arg Pro Glu Ser Lys
           20           25           30
Ala Thr Asn Ala Thr Ile Glu Gly Arg Ser Phe Leu Leu Arg Asn Pro
 35           40           45
Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly Val Asp
 50           55           60
Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro Val Lys
 65           70           75           80
Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg Asp Thr
           85           90           95
Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu Ala Lys
           100          105          110
Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr Asp Asn
           115          120          125
Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu Arg Ile
           130          135          140
Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val Arg Gly
 145          150          155          160
Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys Val Ile
           165          170          175
Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr Arg Ser
           180          185          190
Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile Ile Gln
           195          200          205
Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr Arg Asn
           210          215          220
Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe Thr Phe
 225          230          235          240
Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu Gly Ala
           245          250          255
Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu Leu Ile
           260          265          270
His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn Arg Val
           275          280          285
Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu Glu Val
           290          295          300
Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys Phe Ile
 305          310          315          320
Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn Lys Phe
           325          330          335
Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val Gly Thr
           340          345          350
Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys Tyr Leu
           355          360          365
Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu Lys Phe
           370          375          380
Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp Asn Phe
 385          390          395          400
Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn Phe Asp
           405          410          415
Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr Thr Ile
           420          425          430
Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn Phe Asn

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Li et al., Degradable Clostridial Toxins

		435					440					445				
Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu	Lys	Asn	
		450					455					460				
Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg	Gly	Ile	
465					470					475					480	
Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Asn	Lys	Ala	Leu	
				485					490					495		
Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	
		500						505					510			
Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	
		515					520					525				
Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	
		530				535					540					
Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	
545					550					555					560	
Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	
				565				570						575		
Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	
		580					585						590			
Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	
		595					600					605				
Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	
		610				615					620					
Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	
625					630					635					640	
Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	
				645				650						655		
Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	
		660					665						670			
Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	
		675				680						685				
Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	
		690				695					700					
Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	
705					710					715					720	
Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	
				725				730						735		
Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	
		740					745						750			
Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	
		755				760						765				
Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	
		770				775					780					
Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	
785					790					795					800	
Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	
				805				810						815		
Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	
			820					825					830			
Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	
		835					840					845				
Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	
		850				855					860					
Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	
865					870					875					880	
Asp	Arg	Leu	Lys	Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	
				885					890					895		
Phe	Gln	Leu	Ser	Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	
		900						905					910			

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Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn Leu Arg
    915                      920                      925
Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser Lys Ile
    930                      935                      940
Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn Gln Ile
    945                      950                      955                      960
Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu Lys Asn
    965                      970                      975
Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser Phe Trp
    980                      985                      990
Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn Glu Tyr
    995                      1000                      1005
Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val Ser Leu
    1010                      1015                      1020
Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu Ile Lys
    1025                      1030                      1035                      1040
Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr
    1045                      1050                      1055
Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asn Asn
    1060                      1065                      1070
Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser
    1075                      1080                      1085
Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp
    1090                      1095                      1100
Gly Cys Arg Asp Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu
    1105                      1110                      1115                      1120
Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn
    1125                      1130                      1135
Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln
    1140                      1145                      1150
Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr
    1155                      1160                      1165
Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly
    1170                      1175                      1180
Pro Arg Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu
    1185                      1190                      1195                      1200
Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys
    1205                      1210                      1215
Asp Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val
    1220                      1225                      1230
Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val
    1235                      1240                      1245
Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser
    1250                      1255                      1260
Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys
    1265                      1270                      1275                      1280
Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile
    1285                      1290                      1295
Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp
    1300                      1305                      1310
Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp
    1315                      1320                      1325
Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu Arg Pro Leu
    1330                      1335                      1340

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<210> 87

<211> 1345

<212> PRT

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<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(1345)

<223> BoNT/A-ED-PAR2-Trypsin

<400> 87

Met	Arg	Ser	Pro	Ser	Ala	Ala	Trp	Leu	Leu	Gly	Ala	Ala	Ile	Leu	Leu
1				5				10					15		
Ala	Ala	Ser	Leu	Ser	Cys	Ser	Gly	Thr	Ile	Gln	Gly	Thr	Asn	Arg	Ser
		20					25						30		
Ser	Lys	Gly	Arg	Ser	Leu	Ile	Gly	Lys	Val	Asp	Gly	Thr	Ser	His	Val
	35						40					45			
Thr	Gly	Pro	Phe	Val	Asn	Lys	Gln	Phe	Asn	Tyr	Lys	Asp	Pro	Val	Asn
	50				55						60				
Gly	Val	Asp	Ile	Ala	Tyr	Ile	Lys	Ile	Pro	Asn	Ala	Gly	Gln	Met	Gln
65					70					75					80
Pro	Val	Lys	Ala	Phe	Lys	Ile	His	Asn	Lys	Ile	Trp	Val	Ile	Pro	Glu
				85					90					95	
Arg	Asp	Thr	Phe	Thr	Asn	Pro	Glu	Glu	Gly	Asp	Leu	Asn	Pro	Pro	Pro
			100					105					110		
Glu	Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser
	115						120					125			
Thr	Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe
	130					135					140				
Glu	Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile
145					150					155					160
Val	Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu
				165					170					175	
Lys	Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser
			180					185					190		
Tyr	Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp
	195						200					205			
Ile	Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu
	210					215					220				
Thr	Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp
225					230					235					240
Phe	Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu
				245					250					255	
Leu	Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His
		260						265					270		
Glu	Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro
	275						280					285			
Asn	Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly
	290					295					300				
Leu	Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala
305					310					315					320
Lys	Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr
				325					330					335	
Asn	Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile
				340				345					350		
Val	Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu
	355						360					365			
Lys	Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys
	370					375					380				
Leu	Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu
385					390					395					400
Asp	Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu

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				405					410					415	
Asn	Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn
			420					425					430		
Tyr	Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala
			435					440					445		
Asn	Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys
			450					455				460			
Leu	Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val
465						470				475					480
Arg	Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Asp	Lys	Gly	Tyr	Asn
				485					490						495
Lys	Ala	Leu	Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe
			500					505					510		
Phe	Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu
			515					520					525		
Glu	Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser
			530					535					540		
Leu	Asp	Leu	Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu
545						550					555				560
Pro	Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln
						565					570				575
Leu	Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr
			580					585					590		
Glu	Leu	Asp	Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe
			595					600					605		
Glu	His	Gly	Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala
			610					615					620		
Leu	Leu	Asn	Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val
625						630					635				640
Lys	Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val
						645									655
Glu	Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr
						660							670		
Thr	Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro
						675							685		
Ala	Leu	Asn	Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala
						695							700		
Leu	Ile	Phe	Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile
705						710					715				720
Ala	Ile	Pro	Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn
						725									735
Lys	Val	Leu	Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn
						740							750		
Glu	Lys	Trp	Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala
						755							765		
Lys	Val	Asn	Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala
						775							780		
Leu	Glu	Asn	Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr
785						790							795		800
Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp
						805									815
Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn
						820							830		
Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser
						835							845		
Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu
						855							860		
Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile
865						870									880

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Gly Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr
 885 890 895
 Asp Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu
 900 905 910
 Ser Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu
 915 920 925
 Asn Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala
 930 935 940
 Ser Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys
 945 950 955 960
 Asn Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile
 965 970 975
 Leu Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr
 980 985 990
 Ser Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn
 995 1000 1005
 Asn Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys
 1010 1015 1020
 Val Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln
 1025 1030 1035 1040
 Glu Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile
 1045 1050 1055
 Ser Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg
 1060 1065 1070
 Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys
 1075 1080 1085
 Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met Phe
 1090 1095 1100
 Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp Ile Lys Tyr
 1105 1110 1115 1120
 Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp Leu
 1125 1130 1135
 Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly Asp
 1140 1145 1150
 Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro
 1155 1160 1165
 Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr
 1170 1175 1180
 Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn
 1185 1190 1195 1200
 Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser
 1205 1210 1215
 Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn
 1220 1225 1230
 Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln
 1235 1240 1245
 Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly
 1250 1255 1260
 Asn Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile
 1265 1270 1275 1280
 Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile
 1285 1290 1295
 Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala
 1300 1305 1310
 Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly
 1315 1320 1325
 Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu Arg Pro
 1330 1335 1340
 Leu

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1345

<210> 88
 <211> 1337
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> PEPTIDE
 <222> (1)...(1337)
 <223> BoNT/A-ED-PAR2-Xa

<400> 88
 Met Arg Ser Pro Ser Ala Ala Trp Leu Leu Gly Ala Ala Ile Leu Leu
 1 5 10 15
 Ala Ala Ser Leu Ser Cys Ser Gly Thr Ile Gln Gly Thr Asn Arg Ser
 20 25 30
 Ile Glu Gly Arg Ser Leu Ile Gly Lys Val Pro Phe Val Asn Lys Gln
 35 40 45
 Phe Asn Tyr Lys Asp Pro Val Asn Gly Val Asp Ile Ala Tyr Ile Lys
 50 55 60
 Ile Pro Asn Ala Gly Gln Met Gln Pro Val Lys Ala Phe Lys Ile His
 65 70 75 80
 Asn Lys Ile Trp Val Ile Pro Glu Arg Asp Thr Phe Thr Asn Pro Glu
 85 90 95
 Glu Gly Asp Leu Asn Pro Pro Pro Glu Ala Lys Gln Val Pro Val Ser
 100 105 110
 Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr Asp Asn Glu Lys Asp Asn Tyr
 115 120 125
 Leu Lys Gly Val Thr Lys Leu Phe Glu Arg Ile Tyr Ser Thr Asp Leu
 130 135 140
 Gly Arg Met Leu Leu Thr Ser Ile Val Arg Gly Ile Pro Phe Trp Gly
 145 150 155 160
 Gly Ser Thr Ile Asp Thr Glu Leu Lys Val Ile Asp Thr Asn Cys Ile
 165 170 175
 Asn Val Ile Gln Pro Asp Gly Ser Tyr Arg Ser Glu Glu Leu Asn Leu
 180 185 190
 Val Ile Ile Gly Pro Ser Ala Asp Ile Ile Gln Phe Glu Cys Lys Ser
 195 200 205
 Phe Gly His Glu Val Leu Asn Leu Thr Arg Asn Gly Tyr Gly Ser Thr
 210 215 220
 Gln Tyr Ile Arg Phe Ser Pro Asp Phe Thr Phe Gly Phe Glu Glu Ser
 225 230 235 240
 Leu Glu Val Asp Thr Asn Pro Leu Leu Gly Ala Gly Lys Phe Ala Thr
 245 250 255
 Asp Pro Ala Val Thr Leu Ala His Glu Leu Ile His Ala Gly His Arg
 260 265 270
 Leu Tyr Gly Ile Ala Ile Asn Pro Asn Arg Val Phe Lys Val Asn Thr
 275 280 285
 Asn Ala Tyr Tyr Glu Met Ser Gly Leu Glu Val Ser Phe Glu Glu Leu
 290 295 300
 Arg Thr Phe Gly Gly His Asp Ala Lys Phe Ile Asp Ser Leu Gln Glu
 305 310 315 320
 Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn Lys Phe Lys Asp Ile Ala Ser
 325 330 335
 Thr Leu Asn Lys Ala Lys Ser Ile Val Gly Thr Thr Ala Ser Leu Gln
 340 345 350
 Tyr Met Lys Asn Val Phe Lys Glu Lys Tyr Leu Leu Ser Glu Asp Thr

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		355					360					365			
Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu	Lys	Phe	Asp	Lys	Leu	Tyr	Lys
	370					375					380				
Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp	Asn	Phe	Val	Lys	Phe	Phe	Lys
385					390					395					400
Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn	Phe	Asp	Lys	Ala	Val	Phe	Lys
				405					410					415	
Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr	Thr	Ile	Tyr	Asp	Gly	Phe	Asn
			420					425					430		
Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn	Phe	Asn	Gly	Gln	Asn	Thr	Glu
		435					440					445			
Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu	Lys	Asn	Phe	Thr	Gly	Leu	Phe
	450					455				460					
Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg	Gly	Ile	Ile	Thr	Ser	Lys	Thr
465					470				475						480
Lys	Ser	Leu	Ile	Glu	Gly	Arg	Asn	Lys	Ala	Leu	Asn	Asp	Leu	Cys	Ile
				485					490					495	
Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn	Phe
			500					505					510		
Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn	Ile
		515					520					525			
Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr	Tyr
	530					535					540				
Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu	Asn
545					550					555					560
Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile	Glu
				565					570					575	
Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met	Phe
			580					585					590		
His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile	Ala
		595					600					605			
Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val	Tyr
	610					615					620				
Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr	Glu
625					630					635					640
Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	Thr
				645					650					655	
Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	Thr
			660					665					670		
Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	Leu
		675					680					685			
Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	Ile
	690					695					700				
Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	Phe
705					710					715					720
Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	Ile
				725					730					735	
Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	Lys
			740					745					750		
Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	Leu
		755					760					765			
Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	Thr
	770					775					780				
Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys
785					790					795					800
Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu
				805					810					815	
Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys
			820					825					830		

Li et al., Degradable Clostridial Toxins

Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg
	835						840					845			
Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile
	850						855					860			
Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	Asp
	865						870				875				880
Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	Lys
				885					890					895	
Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile	Lys
			900					905					910		
Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His
	915						920					925			
Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys
	930						935					940			
Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu
	945					950				955					960
Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn
			965						970					975	
Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys
		980						985				990			
Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys
	995						1000					1005			
Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile
	1010						1015				1020				
Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe
	1025					1030				1035					1040
Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile
			1045						1050					1055	
Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	Ile
		1060						1065					1070		
Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	Ile
	1075						1080					1085			
His	Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	Asp	Thr
	1090					1095					1100				
His	Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu	Phe	Asp	Lys	Glu	Leu
	1105				1110				1115						1120
Asn	Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn	Gln	Ser	Asn	Ser	Gly
			1125						1130					1135	
Ile	Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln	Tyr	Asp	Lys	Pro	Tyr
		1140						1145					1150		
Tyr	Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr	Val	Asp	Val	Asn	Asn
	1155						1160					1165			
Val	Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly	Pro	Arg	Gly	Ser	Val
	1170					1175					1180				
Met	Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu	Tyr	Arg	Gly	Thr	Lys
	1185				1190					1195					1200
Phe	Ile	Ile	Lys	Lys	Tyr	Ala	Ser	Gly	Asn	Lys	Asp	Asn	Ile	Val	Arg
			1205						1210					1215	
Asn	Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val	Lys	Asn	Lys	Glu	Tyr
			1220					1225					1230		
Arg	Leu	Ala	Thr	Asn	Ala	Ser	Gln	Ala	Gly	Val	Glu	Lys	Ile	Leu	Ser
	1235						1240					1245			
Ala	Leu	Glu	Ile	Pro	Asp	Val	Gly	Asn	Leu	Ser	Gln	Val	Val	Val	Met
	1250					1255					1260				
Lys	Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys	Cys	Lys	Met	Asn	Leu
	1265				1270					1275					1280
Gln	Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile	Gly	Phe	His	Gln	Phe
			1285						1290					1295	

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Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile
 1300 1305 1310
 Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val
 1315 1320 1325
 Asp Asp Gly Trp Gly Glu Arg Pro Leu
 1330 1335

<210> 89

<211> 1347

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(1347)

<223> BoNT/A-ED-PAR3-Thrombin

<400> 89

Met Lys Ala Leu Ile Phe Ala Ala Ala Gly Leu Leu Leu Leu Leu Pro
 1 5 10 15
 Thr Phe Cys Gln Ser Gly Met Glu Asn Asp Thr Asn Asn Leu Ala Lys
 20 25 30
 Pro Thr Leu Pro Ile Lys Thr Phe Arg Gly Ala Pro Pro Asn Ser Phe
 35 40 45
 Glu Glu Phe Pro Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro
 50 55 60
 Val Asn Gly Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln
 65 70 75 80
 Met Gln Pro Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile
 85 90 95
 Pro Glu Arg Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro
 100 105 110
 Pro Pro Glu Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr
 115 120 125
 Leu Ser Thr Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys
 130 135 140
 Leu Phe Glu Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr
 145 150 155 160
 Ser Ile Val Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr
 165 170 175
 Glu Leu Lys Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp
 180 185 190
 Gly Ser Tyr Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser
 195 200 205
 Ala Asp Ile Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu
 210 215 220
 Asn Leu Thr Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser
 225 230 235 240
 Pro Asp Phe Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn
 245 250 255
 Pro Leu Leu Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu
 260 265 270
 Ala His Glu Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile
 275 280 285
 Asn Pro Asn Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met
 290 295 300
 Ser Gly Leu Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His
 305 310 315 320

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Asp Ala Lys Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr
 325 330 335
 Tyr Tyr Asn Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys
 340 345 350
 Ser Ile Val Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe
 355 360 365
 Lys Glu Lys Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val
 370 375 380
 Asp Lys Leu Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr
 385 390 395 400
 Thr Glu Asp Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr
 405 410 415
 Tyr Leu Asn Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys
 420 425 430
 Val Asn Tyr Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu
 435 440 445
 Ala Ala Asn Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe
 450 455 460
 Thr Lys Leu Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu
 465 470 475 480
 Cys Val Arg Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Asp Lys Gly
 485 490 495
 Tyr Asn Lys Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp Asp
 500 505 510
 Leu Phe Phe Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys
 515 520 525
 Gly Glu Glu Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn
 530 535 540
 Ile Ser Leu Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp
 545 550 555 560
 Asn Glu Pro Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile
 565 570 575
 Gly Gln Leu Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys
 580 585 590
 Lys Tyr Glu Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln
 595 600 605
 Glu Phe Glu His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn
 610 615 620
 Glu Ala Leu Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp
 625 630 635 640
 Tyr Val Lys Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly
 645 650 655
 Trp Val Glu Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val
 660 665 670
 Ser Thr Thr Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile
 675 680 685
 Gly Pro Ala Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val
 690 695 700
 Gly Ala Leu Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro
 705 710 715 720
 Glu Ile Ala Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile
 725 730 735
 Ala Asn Lys Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys
 740 745 750
 Arg Asn Glu Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp
 755 760 765
 Leu Ala Lys Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys
 770 775 780
 Glu Ala Leu Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr

Li *et al.*, Degradable Clostridial Toxins

785				790					795				800		
Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe	Asn
				805					810					815	
Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala	Met
			820					825					830		
Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu	Met
			835				840					845			
Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp	Ala
			850			855					860				
Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly	Thr
865					870					875					880
Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	Asp	Lys	Val	Asn	Asn	Thr	Leu
			885					890						895	
Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	Lys	Tyr	Val	Asp	Asn	Gln	Arg
			900					905					910		
Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	Asn	Thr	Ser
			915				920					925			
Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	Leu	Ser	Arg
			930			935					940				
Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	Asp	Pro	Ile
945					950					955					960
Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu	Ser	Ser	Lys	Ile	Glu
			965					970						975	
Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser	Met	Tyr	Glu	Asn	Phe
			980					985					990		
Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Ser	Ile	Ser
			995				1000					1005			
Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met	Glu	Asn	Asn	Ser	Gly
			1010			1015					1020				
Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile	Trp	Thr	Leu	Gln	Asp
1025					1030					1035					1040
Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys	Tyr	Ser	Gln	Met	Ile
				1045					1050					1055	
Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile	Phe	Val	Thr	Ile	Thr	Asn
			1060					1065					1070		
Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	Ile	Asn	Gly	Arg	Leu	Ile	Asp
			1075				1080					1085			
Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	Ile	His	Ala	Ser	Asn	Asn	Ile
			1090			1095					1100				
Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	Asp	Thr	His	Arg	Tyr	Ile	Trp	Ile
1105					1110					1115					1120
Lys	Tyr	Phe	Asn	Leu	Phe	Asp	Lys	Glu	Leu	Asn	Glu	Lys	Glu	Ile	Lys
			1125					1130						1135	
Asp	Leu	Tyr	Asp	Asn	Gln	Ser	Asn	Ser	Gly	Ile	Leu	Lys	Asp	Phe	Trp
			1140					1145					1150		
Gly	Asp	Tyr	Leu	Gln	Tyr	Asp	Lys	Pro	Tyr	Tyr	Met	Leu	Asn	Leu	Tyr
			1155				1160					1165			
Asp	Pro	Asn	Lys	Tyr	Val	Asp	Val	Asn	Asn	Val	Gly	Ile	Arg	Gly	Tyr
			1170				1175				1180				
Met	Tyr	Leu	Lys	Gly	Pro	Arg	Gly	Ser	Val	Met	Thr	Thr	Asn	Ile	Tyr
1185					1190					1195					1200
Leu	Asn	Ser	Ser	Leu	Tyr	Arg	Gly	Thr	Lys	Phe	Ile	Ile	Lys	Lys	Tyr
			1205					1210						1215	
Ala	Ser	Gly	Asn	Lys	Asp	Asn	Ile	Val	Arg	Asn	Asn	Asp	Arg	Val	Tyr
			1220					1225					1230		
Ile	Asn	Val	Val	Val	Lys	Asn	Lys	Glu	Tyr	Arg	Leu	Ala	Thr	Asn	Ala
			1235				1240					1245			
Ser	Gln	Ala	Gly	Val	Glu	Lys	Ile	Leu	Ser	Ala	Leu	Glu	Ile	Pro	Asp
			1250				1255				1260				

Li *et al.*, Degradable Clostridial Toxins

Val Gly Asn Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp Gln
 1265 1270 1275 1280
 Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn
 1285 1290 1295
 Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys Leu
 1300 1305 1310
 Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg Thr
 1315 1320 1325
 Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly Glu
 1330 1335 1340
 Arg Pro Leu
 1345

<210> 90
 <211> 1339
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> PEPTIDE
 <222> (1)...(1339)
 <223> BoNT/A-ED-PAR3-Xa

<400> 90
 Met Lys Ala Leu Ile Phe Ala Ala Ala Gly Leu Leu Leu Leu Leu Pro
 1 5 10 15
 Thr Phe Cys Gln Ser Gly Met Glu Asn Asp Thr Asn Asn Leu Ala Lys
 20 25 30
 Pro Thr Ile Glu Gly Arg Thr Phe Arg Gly Ala Pro Pro Phe Val Asn
 35 40 45
 Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly Val Asp Ile Ala Tyr
 50 55 60
 Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro Val Lys Ala Phe Lys
 65 70 75 80
 Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg Asp Thr Phe Thr Asn
 85 90 95
 Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu Ala Lys Gln Val Pro
 100 105 110
 Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr Asp Asn Glu Lys Asp
 115 120 125
 Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu Arg Ile Tyr Ser Thr
 130 135 140
 Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val Arg Gly Ile Pro Phe
 145 150 155 160
 Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys Val Ile Asp Thr Asn
 165 170 175
 Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr Arg Ser Glu Glu Leu
 180 185 190
 Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile Ile Gln Phe Glu Cys
 195 200 205
 Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr Arg Asn Gly Tyr Gly
 210 215 220
 Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe Thr Phe Gly Phe Glu
 225 230 235 240
 Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu Gly Ala Gly Lys Phe
 245 250 255
 Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu Leu Ile His Ala Gly
 260 265 270

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His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn	Arg	Val	Phe	Lys	Val
		275					280					285			
Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu	Glu	Val	Ser	Phe	Glu
		290				295					300				
Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys	Phe	Ile	Asp	Ser	Leu
305					310					315					320
Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn	Lys	Phe	Lys	Asp	Ile
				325					330					335	
Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val	Gly	Thr	Thr	Ala	Ser
			340					345					350		
Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys	Tyr	Leu	Leu	Ser	Glu
		355					360					365			
Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu	Lys	Phe	Asp	Lys	Leu
		370				375					380				
Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp	Asn	Phe	Val	Lys	Phe
385					390					395					400
Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn	Phe	Asp	Lys	Ala	Val
				405					410					415	
Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr	Thr	Ile	Tyr	Asp	Gly
			420					425					430		
Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn	Phe	Asn	Gly	Gln	Asn
		435					440					445			
Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu	Lys	Asn	Phe	Thr	Gly
		450				455					460				
Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg	Gly	Ile	Ile	Thr	Ser
465					470				475						480
Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Asn	Lys	Ala	Leu	Asn	Asp	Leu
				485					490					495	
Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp
			500				505						510		
Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr
		515					520					525			
Asn	Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln
		530				535					540				
Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile
545					550					555					560
Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn
				565					570					575	
Ile	Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr
			580					585					590		
Met	Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg
		595					600					605			
Ile	Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg
		610				615					620				
Val															

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				740				745					750			
Tyr	Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	
		755					760					765				
Asp	Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	
	770					775					780					
Ala	Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	
785					790					795					800	
Glu	Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	
			805						810					815		
Asn	Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	
			820					825					830			
Gln	Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	
		835					840					845				
Lys	Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	
	850					855					860					
Tyr	Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	
865					870					875					880	
Lys	Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	
				885					890					895		
Ser	Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	
			900					905					910			
Ile	Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	
		915					920					925				
Asn	His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	
	930					935					940					
Ser	Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	
945					950					955					960	
Asn	Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	
				965					970					975		
Tyr	Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	
			980					985					990			
Pro	Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	
		995					1000					1005				
Asn	Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	
	1010					1015					1020					
Glu	Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	
1025					1030					1035					1040	
Val	Phe	Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	
				1045					1050					1055		
Trp	Ile	Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	
			1060					1065					1070			
Tyr	Ile	Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	

Li *et al.*, Degradable Clostridial Toxins

Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys
 1220 1225 1230
 Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile
 1235 1240 1245
 Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val
 1250 1255 1260
 Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met
 1265 1270 1275 1280
 Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His
 1285 1290 1295
 Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg
 1300 1305 1310
 Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile
 1315 1320 1325
 Pro Val Asp Asp Gly Trp Gly Glu Arg Pro Leu
 1330 1335

<210> 91

<211> 1356

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(1356)

<223> BoNT/A-ED-PAR4-Thrombin

<400> 91

Met Trp Gly Arg Leu Leu Leu Trp Pro Leu Val Leu Gly Phe Ser Leu
 1 5 10 15
 Ser Gly Gly Thr Gln Thr Pro Ser Val Tyr Asp Glu Ser Gly Ser Thr
 20 25 30
 Gly Gly Gly Asp Asp Ser Thr Pro Ser Ile Leu Pro Ala Pro Arg Gly
 35 40 45
 Tyr Pro Gly Gln Val Cys Ala Asn Asp Ser Asp Thr Leu Pro Phe Val
 50 55 60
 Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly Val Asp Ile Ala
 65 70 75 80
 Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro Val Lys Ala Phe
 85 90 95
 Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg Asp Thr Phe Thr
 100 105 110
 Asn Pro Glu Gly Asp Leu Asn Pro Pro Pro Glu Ala Lys Gln Val
 115 120 125
 Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr Asp Asn Glu Lys
 130 135 140
 Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu Arg Ile Tyr Ser
 145 150 155 160
 Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val Arg Gly Ile Pro
 165 170 175
 Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys Val Ile Asp Thr
 180 185 190
 Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr Arg Ser Glu Glu
 195 200 205
 Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile Ile Gln Phe Glu
 210 215 220
 Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr Arg Asn Gly Tyr
 225 230 235 240

Li et al., Degradable Clostridial Toxins

Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe	Thr	Phe	Gly	Phe
				245					250					255	
Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu	Gly	Ala	Gly	Lys
			260					265					270		
Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu	Leu	Ile	His	Ala
			275					280					285		
Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn	Arg	Val	Phe	Lys
			290			295					300				
Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu	Glu	Val	Ser	Phe
305					310					315					320
Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys	Phe	Ile	Asp	Ser
				325					330					335	
Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn	Lys	Phe	Lys	Asp
			340					345					350		
Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val	Gly	Thr	Thr	Ala
			355				360					365			
Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys	Tyr	Leu	Leu	Ser
			370			375					380				
Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu	Lys	Phe	Asp	Lys
385					390					395					400
Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp	Asn	Phe	Val	Lys
				405					410					415	
Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn	Phe	Asp	Lys	Ala
			420					425					430		
Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr	Thr	Ile	Tyr	Asp
			435				440					445			
Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn	Phe	Asn	Gly	Gln
			450			455					460				
Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu	Lys	Asn	Phe	Thr
465					470					475					480
Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg	Gly	Ile	Ile	Thr
				485					490					495	
Ser	Lys	Thr	Lys	Ser	Leu	Asp	Lys	Gly	Tyr	Asn	Lys	Ala	Leu	Asn	Asp
			500					505					510		
Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu
			515				520					525			
Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp
			530			535					540				
Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln
545					550					555					560
Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser
				565					570					575	
Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro
			580					585					590		
Asn															

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705					710					715				720	
Ala	Val	Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu
				725					730					735	
Gly	Thr	Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val
			740					745					750		
Gln	Thr	Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu
		755				760					765				
Val	Tyr	Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln
	770				775						780				
Ile	Asp	Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala
785				790						795					800
Glu	Ala	Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu
				805					810					815	
Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys
			820					825					830		
Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu
		835					840					845			
Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly
	850					855					860				
Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu
865				870						875					880
Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg
				885					890					895	
Leu	Lys	Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln
		900						905					910		
Leu	Ser	Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu
	915						920					925			
Tyr	Ile	Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu
	930					935					940				
Ser	Asn	His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile
945				950						955					960
Gly	Ser	Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu
				965					970					975	
Phe	Asn	Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile
			980					985					990		
Val	Tyr	Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg
	995					1000						1005			
Ile	Pro	Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile
	1010					1015						1020			
Ile	Asn	Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr
1025				1030						1035					1040
Gly	Glu	Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg
				1045					1050					1055	
Val	Val	Phe	Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn
			1060					1065					1070		
Arg	Trp	Ile	Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys
	1075					1080						1085			
Ile	Tyr	Ile	Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu
	1090					1095					1100				
Gly	Asn	Ile	His	Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys
1105				1110						1115					1120
Arg	Asp	Thr	His	Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu	Phe	Asp
				1125					1130					1135	
Lys	Glu	Leu	Asn	Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn	Gln	Ser
			1140					1145					1150		
Asn	Ser	Gly	Ile	Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln	Tyr	Asp
	1155						1160					1165			

Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp

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1170	1175	1180
Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg		
1185	1190	1195
Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg		1200
	1205	1210
Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn		1215
	1220	1225
Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn		1230
	1235	1240
Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys		1245
	1250	1255
Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val		1260
1265	1270	1275
Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys		1280
	1285	1290
Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe		1295
	1300	1305
His Gln Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn		1310
	1315	1320
Arg Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe		1325
	1330	1335
Ile Pro Val Asp Asp Gly Trp Gly Glu Arg Pro Leu		1340
1345	1350	1355

<210> 92

<211> 1348

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(1348)

<223> BoNT/A-ED-PAR4-Xa

<400> 92

Met Trp Gly Arg Leu Leu Leu Trp Pro Leu Val Leu Gly Phe Ser Leu	
1 5 10 15	
Ser Gly Gly Thr Gln Thr Pro Ser Val Tyr Asp Glu Ser Gly Ser Thr	
20 25 30	
Gly Gly Gly Asp Asp Ser Thr Pro Ser Ile Leu Ile Glu Gly Arg Gly	
35 40 45	
Tyr Pro Gly Gln Val Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp	
50 55 60	
Pro Val Asn Gly Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly	
65 70 75 80	
Gln Met Gln Pro Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val	
85 90 95	
Ile Pro Glu Arg Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn	
100 105 110	
Pro Pro Pro Glu Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr	
115 120 125	
Tyr Leu Ser Thr Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr	
130 135 140	
Lys Leu Phe Glu Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu	
145 150 155 160	
Thr Ser Ile Val Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp	
165 170 175	
Thr Glu Leu Lys Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro	

Li *et al.*, Degradable Clostridial Toxins

			180					185					190			
Asp	Gly	Ser	Tyr	Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	
		195					200					205				
Ser	Ala	Asp	Ile	Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	
	210					215					220					
Leu	Asn	Leu	Thr	Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	
225					230					235				240		
Ser	Pro	Asp	Phe	Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	
				245						250				255		
Asn	Pro	Leu	Leu	Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	
			260					265					270			
Leu	Ala	His	Glu	Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	
		275					280					285				
Ile	Asn	Pro	Asn	Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	
	290					295					300					
Met	Ser	Gly	Leu	Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	
305					310					315				320		
His	Asp	Ala	Lys	Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	
				325					330					335		
Tyr	Tyr	Tyr	Asn	Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	
			340					345					350			
Lys	Ser	Ile	Val	Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	
		355					360					365				
Phe	Lys	Glu	Lys	Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	
	370				375						380					
Val	Asp	Lys	Leu	Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	
385					390					395				400		
Tyr	Thr	Glu	Asp	Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	
				405					410					415		
Thr	Tyr	Leu	Asn	Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	
			420					425					430			
Lys	Val	Asn	Tyr	Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	
		435					440					445				
Leu	Ala	Ala	Asn	Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	
	450					455					460					
Phe	Thr	Lys	Leu	Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	
465					470					475				480		
Leu	Cys	Val	Arg	Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	
				485					490					495		
Gly	Arg	Asn	Lys	Ala	Leu	Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp	
			500					505					510			
Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	
		515					520					525				
Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu	
	530					53										

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Gly Trp Val Glu Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu
 660 665 670
 Val Ser Thr Thr Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr
 675 680 685
 Ile Gly Pro Ala Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe
 690 695 700
 Val Gly Ala Leu Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile
 705 710 715 720
 Pro Glu Ile Ala Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr
 725 730 735
 Ile Ala Asn Lys Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser
 740 745 750
 Lys Arg Asn Glu Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn
 755 760 765
 Trp Leu Ala Lys Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met
 770 775 780
 Lys Glu Ala Leu Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn
 785 790 795 800
 Tyr Gln Tyr Asn Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe
 805 810 815
 Asn Ile Asp Asp Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala
 820 825 830
 Met Ile Asn Ile Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu
 835 840 845
 Met Asn Ser Met Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp
 850 855 860
 Ala Ser Leu Lys Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly
 865 870 875 880
 Thr Leu Ile Gly Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr
 885 890 895
 Leu Ser Thr Asp Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln
 900 905 910
 Arg Leu Leu Ser Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr
 915 920 925
 Ser Ile Leu Asn Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser
 930 935 940
 Arg Tyr Ala Ser Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro
 945 950 955 960
 Ile Asp Lys Asn Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile
 965 970 975
 Glu Val Ile Leu Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn
 980 985 990
 Phe Ser Thr Ser Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile
 995 1000 1005
 Ser Leu Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser
 1010 1015 1020
 Gly Trp Lys Val Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln
 1025 1030 1035 1040
 Asp Thr Gln Glu Ile Lys Gln Arg Val Val Phe Lys Tyr Ser Gln Met
 1045 1050 1055
 Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr
 1060 1065 1070
 Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile
 1075 1080 1085
 Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn
 1090 1095 1100
 Ile Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp
 1105 1110 1115 1120
 Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile

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      1125      1130      1135
Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe
      1140      1145      1150
Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu
      1155      1160      1165
Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly
      1170      1175      1180
Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile
1185      1190      1195      1200
Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys
      1205      1210      1215
Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val
      1220      1225      1230
Tyr Ile Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn
      1235      1240      1245
Ala Ser Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro
      1250      1255      1260
Asp Val Gly Asn Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp
1265      1270      1275      1280
Gln Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly
      1285      1290      1295
Asn Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys
      1300      1305      1310
Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg
      1315      1320      1325
Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly
      1330      1335      1340
Glu Arg Pro Leu
1345

```

<210> 93

<211> 1306

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(1306)

<223> BoNT/A-TD-PAR1-Thrombin

<400> 93

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Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1      5      10      15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
      20      25      30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
      35      40      45
Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
      50      55      60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
      65      70      75      80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
      85      90      95
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
      100      105      110
Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
      115      120      125
Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr

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Li et al., Degradable Clostridial Toxins

130	135	140
Arg Ser Glu Glu Leu Asn	Leu Val Ile Ile Gly	Pro Ser Ala Asp Ile
145	150	155
Ile Gln Phe Glu Cys Lys	Ser Phe Gly His Glu	Val Leu Asn Leu Thr
165	170	175
Arg Asn Gly Tyr Gly Ser	Thr Gln Tyr Ile Arg	Phe Ser Pro Asp Phe
180	185	190
Thr Phe Gly Phe Glu Glu	Ser Leu Glu Val Asp	Thr Asn Pro Leu Leu
195	200	205
Gly Ala Gly Lys Phe Ala	Thr Asp Pro Ala Val	Thr Leu Ala His Glu
210	215	220
Leu Ile His Ala Gly His	Arg Leu Tyr Gly Ile	Ala Ile Asn Pro Asn
225	230	235
Arg Val Phe Lys Val Asn	Thr Asn Ala Tyr Tyr	Glu Met Ser Gly Leu
245	250	255
Glu Val Ser Phe Glu Glu	Leu Arg Thr Phe Gly	Gly His Asp Ala Lys
260	265	270
Phe Ile Asp Ser Leu Gln	Glu Asn Glu Phe Arg	Leu Tyr Tyr Tyr Asn
275	280	285
Lys Phe Lys Asp Ile Ala	Ser Thr Leu Asn Lys	Ala Lys Ser Ile Val
290	295	300
Gly Thr Thr Ala Ser Leu	Gln Tyr Met Lys Asn	Val Phe Lys Glu Lys
305	310	315
Tyr Leu Leu Ser Glu Asp	Thr Ser Gly Lys Phe	Ser Val Asp Lys Leu
325	330	335
Lys Phe Asp Lys Leu Tyr	Lys Met Leu Thr Glu	Ile Tyr Thr Glu Asp
340	345	350
Asn Phe Val Lys Phe Phe	Lys Val Leu Asn Arg	Lys Thr Tyr Leu Asn
355	360	365
Phe Asp Lys Ala Val Phe	Lys Ile Asn Ile Val	Pro Lys Val Asn Tyr
370	375	380
Thr Ile Tyr Asp Gly Phe	Asn Leu Arg Asn Thr	Asn Leu Ala Ala Asn
385	390	395
Phe Asn Gly Gln Asn Thr	Glu Ile Asn Asn Met	Asn Phe Thr Lys Leu
405	410	415
Lys Asn Phe Thr Gly Leu	Phe Glu Phe Tyr Lys	Leu Leu Cys Val Arg
420	425	430
Gly Ile Ile Thr Ser Lys	Thr Lys Ser Leu Pro	Arg Ser Phe Leu Leu
435	440	445
Arg Asn Pro Asn Asp Lys	Tyr Glu Pro Phe Ala	Leu Asn Asp Leu Cys
450	455	460
Ile Lys Val Asn Asn Trp	Asp Leu Phe Phe Ser	Pro Ser Glu Asp Asn
465	470	475
Phe Thr Asn Asp Leu Asn	Lys Gly Glu Glu Ile	Thr Ser Asp Thr Asn
485	490	495
Ile Glu Ala Ala Glu Glu	Asn Ile Ser Leu Asp	Leu Ile Gln Gln Tyr
500	505	510
Tyr Leu Thr Phe Asn Phe	Asp Asn Glu Pro Glu	Asn Ile Ser Ile Glu
515	520	525
Asn Leu Ser Ser Asp Ile	Ile Ile Gly Gln Leu	Glu Leu Met Pro Asn Ile
530	535	540
Glu Arg Phe Pro Asn Gly	Lys Lys Tyr Glu Leu	Asp Lys Tyr Thr Met
545	550	555
Phe His Tyr Leu Arg Ala	Gln Glu Phe Glu His	Gly Lys Ser Arg Ile
565	570	575
Ala Leu Thr Asn Ser Val	Asn Glu Ala Leu Leu	Asn Pro Ser Arg Val
580	585	590
Tyr Thr Phe Ser Ser Asp	Tyr Val Lys Lys Val	Asn Lys Ala Thr
595	600	605

Li *et al.*, Degradable Clostridial Toxins

Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	610	615	620
Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	625	630	635
Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	645	650	655
Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	660	665	670
Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	675	680	685
Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	690	695	700
Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	705	710	715
Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	725	730	735
Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	740	745	750
Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	755	760	765
Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	770	775	780
Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	785	790	795
Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	805	810	815
Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	820	825	830
Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	835	840	845
Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	850	855	860
Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile	865	870	875
Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	885	890	895
His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	900	905	910
Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	915	920	925
Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	930	935	940
Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	945	950	955
Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	965	970	975
Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	980	985	990
Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	995	1000	1005
Phe	Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	1010	1015	1020
Ile	Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	1025	1030	1035
Ile	Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	1045	1050	1055
Ile	His	Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	Asp	1060	1065	1070

Li *et al.*, Degradable Clostridial Toxins

Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu
 1075 1080 1085
 Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser
 1090 1095 1100
 Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro
 1105 1110 1115 1120
 Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn
 1125 1130 1135
 Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser
 1140 1145 1150
 Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr
 1155 1160 1165
 Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val
 1170 1175 1180
 Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys Glu
 1185 1190 1195 1200
 Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile Leu
 1205 1210 1215
 Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val Val
 1220 1225 1230
 Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met Asn
 1235 1240 1245
 Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His Gln
 1250 1255 1260
 Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln
 1265 1270 1275 1280
 Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro
 1285 1290 1295
 Val Asp Asp Gly Trp Gly Glu Arg Pro Leu
 1300 1305

<210> 94
 <211> 1300
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> PEPTIDE
 <222> (1)...(1300)
 <223> BoNT/A-TD-PAR1-Xa

<400> 94
 Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1 5 10 15
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
 20 25 30
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
 35 40 45
 Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
 50 55 60
 Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
 65 70 75 80
 Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
 85 90 95
 Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
 100 105 110
 Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys

Li et al., Degradable Clostridial Toxins

		115					120					125				
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr	
	130					135					140					
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile	
145					150					155					160	
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr	
				165					170					175		
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe	
			180					185					190			
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu	
		195					200					205				
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu	
	210					215					220					
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn	
225					230					235					240	
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu	
				245					250					255		
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys	
			260					265					270			
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn	
		275					280					285				
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val	
	290					295					300					
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys	
305					310					315					320	
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu	
				325					330					335		
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp	
			340					345					350			
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn	
		355					360					365				
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr	
	370					375					380					
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn	
385					390					395					400	
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu	
				405					410					415		
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg	
			420					425					430			
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Ser	Phe	
		435					440					445				
Leu	Leu	Arg	Asn	Ala	Leu	Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp	
		450				455					460					
Asp	Leu</															

Li et al., Degradable Clostridial Toxins

										580						585						590						
Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe	Leu													
										595			600			605												
Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser	Glu													
										610			615			620												
Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro	Tyr													
										625			630			635												
Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	Asp	Phe													
										645			650			655												
Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	Phe	Ile													
										660			665			670												
Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	Ser	Tyr													
										675			680			685												
Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	Leu	Ser													
										690			695			700												
Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	Thr	Asn													
										705			710			715												
Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	Lys	Met													
										725			730			735												
Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	Ile	Asn													
										740			745			750												
Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe													
										755			760			765												
Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala													
										770			775			780												
Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu													
										785			790			795												
Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp													
										805			810			815												
Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly													
										820			825			830												
Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	Asp	Lys	Val	Asn	Asn	Thr													
										835			840			845												
Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	Lys	Tyr	Val	Asp	Asn	Gln													
										850			855			860												
Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	Asn	Thr													
										865			870			875												
Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	Leu	Ser													
										885			890			895												
Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	Asp	Pro													
										900			905			910												
Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu	Ser	Ser	Lys	Ile													
										915			920			925												
Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser	Met	Tyr	Glu	Asn													
										930			935			940												
Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Ser	Ile													
										945			950			955												
Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met	Glu	Asn	Asn	Ser													
										965			970			975												
Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile	Trp	Thr	Leu	Gln													
										980			985			990												
Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys	Tyr	Ser	Gln	Met													
										995			1000			1005												
Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile	Phe	Val	Thr	Ile	Thr													
										1010			1015			1020												
Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	Ile	Asn	Gly	Arg	Leu	Ile													
										1025			1030			1035												
Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	Ile	His	Ala	Ser	Asn	Asn													

Li *et al.*, Degradable Clostridial Toxins

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                1045                1050                1055
Ile Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp
                1060                1065                1070
Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile
                1075                1080                1085
Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe
                1090                1095                1100
Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu
1105                1110                1115                1120
Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly
                1125                1130                1135
Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile
                1140                1145                1150
Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys
                1155                1160                1165
Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val
                1170                1175                1180
Tyr Ile Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn
1185                1190                1195                1200
Ala Ser Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro
                1205                1210                1215
Asp Val Gly Asn Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp
                1220                1225                1230
Gln Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly
                1235                1240                1245
Asn Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys
                1250                1255                1260
Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg
1265                1270                1275                1280
Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly
                1285                1290                1295
Glu Arg Pro Leu
                1300

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<210> 95

<211> 1306

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(1306)

<223> BoNT/A-TD-PAR2-Trypsin

<400> 95

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Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1                5                10                15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
                20                25                30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
                35                40                45
Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
                50                55                60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
65                70                75                80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
                85                90                95
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val

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Li et al., Degradable Clostridial Toxins

			100					105					110			
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys	
		115					120					125				
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr	
	130					135					140					
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile	
145					150					155					160	
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr	
				165					170						175	
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe	
			180					185					190			
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu	
		195					200					205				
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu	
	210					215					220					
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn	
225					230					235					240	
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu	
				245					250					255		
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys	
			260					265					270			
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn	
		275					280					285				
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val	
	290					295					300					
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys	
305					310					315					320	
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu	
				325					330					335		
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp	
			340					345					350			
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn	
		355					360					365				
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr	
	370					375					380					
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn	
385					390					395					400	
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu	
				405					410					415		
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg	
			420					425					430			
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Gly	Arg	Ser	Leu	Ile	Gly	
	435						440					445				
Lys	Val	Asp	Gly	Thr	Ser	His	Val	Thr	Gly	Ala	Leu	Asn	Asp	Leu	Cys	
	450					455										

Li *et al.*, Degradable Clostridial Toxins

Ala Leu Thr Asn Ser Val Asn Glu Ala Leu Leu Asn Pro Ser Arg Val
 580 585 590
 Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys Lys Val Asn Lys Ala Thr
 595 600 605
 Glu Ala Ala Met Phe Leu Gly Trp Val Glu Gln Leu Val Tyr Asp Phe
 610 615 620
 Thr Asp Glu Thr Ser Glu Val Ser Thr Thr Asp Lys Ile Ala Asp Ile
 625 630 635 640
 Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly Asn Met
 645 650 655
 Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu Ile Phe Ser Gly Ala Val
 660 665 670
 Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala Ile Pro Val Leu Gly Thr
 675 680 685
 Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys Val Leu Thr Val Gln Thr
 690 695 700
 Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu Lys Trp Asp Glu Val Tyr
 705 710 715 720
 Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys Val Asn Thr Gln Ile Asp
 725 730 735
 Leu Ile Arg Lys Lys Met Lys Glu Ala Leu Glu Asn Gln Ala Glu Ala
 740 745 750

 Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn Gln Tyr Thr Glu Glu Glu
 755 760 765
 Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp Leu Ser Ser Lys Leu Asn
 770 775 780
 Glu Ser Ile Asn Lys Ala Met Ile Asn Ile Asn Lys Phe Leu Asn Gln
 785 790 795 800
 Cys Ser Val Ser Tyr Leu Met Asn Ser Met Ile Pro Tyr Gly Val Lys
 805 810 815
 Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys Asp Ala Leu Leu Lys Tyr
 820 825 830
 Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly Gln Val Asp Arg Leu Lys
 835 840 845
 Asp Lys Val Asn Asn Thr Leu Ser Thr Asp Ile Pro Phe Gln Leu Ser
 850 855 860
 Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser Thr Phe Thr Glu Tyr Ile
 865 870 875 880
 Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn Leu Arg Tyr Glu Ser Asn
 885 890 895
 His Leu Ile Asp Leu Ser Arg Tyr Ala Ser Lys Ile Asn Ile Gly Ser
 900 905 910
 Lys Val Asn Phe Asp Pro Ile Asp Lys Asn Gln Ile Gln Leu Phe Asn
 915 920 925
 Leu Glu Ser Ser Lys Ile Glu Val Ile Leu Lys Asn Ala Ile Val Tyr
 930 935 940
 Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser Phe Trp Ile Arg Ile Pro
 945 950 955 960
 Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn Glu Tyr Thr Ile Ile Asn
 965 970 975
 Cys Met Glu Asn Asn Ser Gly Trp Lys Val Ser Leu Asn Tyr Gly Glu
 980 985 990
 Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu Ile Lys Gln Arg Val Val
 995 1000 1005
 Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp
 1010 1015 1020
 Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr
 1025 1030 1035 1040

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Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn
      1045                      1050                      1055
Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp Gly Cys Arg Asp
      1060                      1065                      1070
Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu
      1075                      1080                      1085
Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser
      1090                      1095                      1100
Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro
1105                      1110                      1115                      1120
Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn
      1125                      1130                      1135
Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser
      1140                      1145                      1150
Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr
      1155                      1160                      1165
Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val
      1170                      1175                      1180
Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys Glu
1185                      1190                      1195                      1200
Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile Leu
      1205                      1210                      1215
Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val Val
      1220                      1225                      1230
Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met Asn
      1235                      1240                      1245
Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His Gln
      1250                      1255                      1260
Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln
1265                      1270                      1275                      1280
Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro
      1285                      1290                      1295
Val Asp Asp Gly Trp Gly Glu Arg Pro Leu
      1300                      1305

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<210> 96

<211> 1300

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(1300)

<223> BoNT/A-TD-PAR2-Xa

<400> 96

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Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
  1              5              10              15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
      20              25              30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
      35              40              45
Asp Thr Phe Thr Asn Pro Glu Gly Asp Leu Asn Pro Pro Pro Glu
      50              55              60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
      65              70              75              80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu

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Li *et al.*, Degradable Clostridial Toxins

				85					90				95				
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val		
			100					105					110				
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys		
		115					120					125					
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr		
		130				135					140						
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile		
145					150					155					160		
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr		
				165				170						175			
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe		
		180						185					190				
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu		
		195					200					205					
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu		
	210					215					220						
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn		
225					230					235					240		
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu		
				245				250						255			
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys		
		260						265					270				
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn		
	275						280					285					
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val		
	290					295				300							
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys		
305					310					315					320		
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu		
			325					330						335			
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp		
		340					345					350					
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn		
	355					360						365					
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr		
	370					375				380							
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn		
385					390					395					400		
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu		
			405					410						415			
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg		
		420					425					430					
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Ser	Leu		
	435						440					445					
Ile	Gly	Lys	Val	Ala	Leu	Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp		
	450					455				460							
Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn		
465					470					475					480		
Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu		
			485					490						495			
Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe		
		500						505					510				
Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile		
	515						520					525					
Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly		
	530					535				540							
Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu	Arg	Ala		

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545					550					555				560
Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	Ser
				565					570					575
Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe	Ser
			580						585				590	
Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe
		595					600					605		Leu
Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser
	610						615				620			Glu
Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro
625					630					635				640
Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	Asp
				645				650					655	Phe
Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	Phe
		660					665					670		Ile
Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	Ser
	675						680					685		Tyr
Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	Leu
	690					695				700				Ser
Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	Thr
705					710					715				Asn
Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	Lys
			725						730					Met
Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	Asn
		740					745					750		
Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	Asn
	755					760					765			Phe
Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys
	770					775				780				Ala
Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr
785					790					795				Leu
Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe
			805						810					Asp
Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg
		820						825				830		Gly
Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	Asp	Lys	Val	Asn	Asn
	835						840					845		Thr
Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	Lys	Tyr	Val	Asp	Asn
	850					855				860				Gln
Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	Asn
865					870					875				Thr
Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	Leu
			885						890					Ser
Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	Asp
	900							905				910		Pro
Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu	Ser	Ser	Lys
	915						920					925		Ile
Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser	Met	Tyr	Glu
	930					935				940				Asn
Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Ser
945					950					955				Ile
Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met	Glu	Asn	Asn
			965						970					Ser
Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile	Trp	Thr	Leu
		980						985					990	Gln
Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys	Tyr	Ser	Gln
	995						1000					1005		Met
Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile	Phe	Val	Thr	Ile

Li et al., Degradable Clostridial Toxins

1010	1015	1020
Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile		
1025	1030	1035
Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn		1040
	1045	1050
Ile Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp		1055
	1060	1065
Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile		1070
	1075	1080
Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe		1085
	1090	1095
Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu		1100
1105	1110	1115
Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly		1120
	1125	1130
Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile		1135
	1140	1145
Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys		1150
	1155	1160
Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val		1165
	1170	1175
Tyr Ile Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn		1180
1185	1190	1195
Ala Ser Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro		1200
	1205	1210
Asp Val Gly Asn Leu Ser Gln Val Val Met Lys Ser Lys Asn Asp		1215
	1220	1225
Gln Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly		1230
	1235	1240
Asn Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys		1245
	1250	1255
Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg		1260
1265	1270	1275
Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly		1280
	1285	1290
		1295
Glu Arg Pro Leu		
1300		

<210> 97

<211> 1306

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(1306)

<223> BoNT/A-TD-PAR3-Thrombin

<400> 97

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly	
1	5
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro	
	20
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg	
	35
Asp Thr Phe Thr Asn Pro Glu Gly Asp Leu Asn Pro Pro Pro Glu	
	50
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr	
	55
	60

Li *et al.*, Degradable Clostridial Toxins

65					70					75					80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
		115				120					125				
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130				135				140						
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150				155					160	
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
			165					170					175		
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
		180						185				190			
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
	195					200					205				
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210				215					220					
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225				230				235					240		
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
			245					250				255			
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
		260					265					270			
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
	275					280					285				
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
	290				295				300						
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305				310					315					320	
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
			325					330					335		
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
		340					345					350			
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
	355				360						365				
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370				375				380						
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385				390					395					400	
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
			405					410					415		
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
		420					425					430			
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Lys	Thr	Phe	Arg	Gly
	435					440					445				
Ala	Pro	Pro	Asn	Ser	Phe	Glu	Glu	Phe	Pro	Ala	Leu	Asn	Asp	Leu	Cys
	450				455				460						
Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn
465				470					475					480	
Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn
			485					490					495		
Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr
	500					505					510				
Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu
	515					520					525				
Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile

Li *et al.*, Degradable Clostridial Toxins

530						535					540				
Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met
545					550					555					560
Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile
				565					570						575
Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val
			580					585					590		
Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr
		595					600					605			
Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe
	610					615					620				
Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile
625					630					635					640
Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met
				645					650					655	
Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val
			660					665					670		
Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr
		675					680					685			
Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr
	690					695					700				
Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr
705					710					715					720
Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp
				725					730					735	
Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala
			740					745					750		
Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu
		755					760					765			
Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn
	770					775					780				
Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln
785					790					795					800
Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys
				805					810					815	
Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr
			820					825					830		
Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys
		835				840						845			
Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser
	850					855					860				
Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile
865					870					875					880
Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn
				885					890					895	
His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser
			900					905					910		
Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn
		915					920					925			
Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr
	930					935					940				
Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro
945					950					955					960
Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn
				965					970					975	
Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu
			980					985					990		
Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val
		995					1000					1005			

Li et al., Degradable Clostridial Toxins

Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp
 1010 1015 1020
 Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr
 1025 1030 1035 1040
 Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn
 1045 1050 1055
 Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp Gly Cys Arg Asp
 1060 1065 1070
 Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu
 1075 1080 1085
 Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser
 1090 1095 1100
 Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro
 1105 1110 1115 1120
 Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn
 1125 1130 1135
 Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser
 1140 1145 1150
 Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr
 1155 1160 1165
 Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val
 1170 1175 1180
 Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys Glu
 1185 1190 1195 1200
 Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile Leu
 1205 1210 1215
 Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val Val
 1220 1225 1230
 Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met Asn
 1235 1240 1245
 Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His Gln
 1250 1255 1260
 Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln
 1265 1270 1275 1280
 Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro
 1285 1290 1295
 Val Asp Asp Gly Trp Gly Glu Arg Pro Leu
 1300 1305

<210> 98

<211> 1300

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(1300)

<223> BoNT/A-TD-PAR3-Xa

<400> 98

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1 5 10 15
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
 20 25 30
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
 35 40 45
 Asp Thr Phe Thr Asn Pro Glu Gly Asp Leu Asn Pro Pro Pro Glu
 50 55 60

Li et al., Degradable Clostridial Toxins

Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser	Thr
65					70					75					80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
		115					120					125			
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130					135					140				
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150					155					160
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
				165					170						175
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
			180					185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
		195					200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215					220				
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225					230					235					240
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
				245					250					255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
			260					265					270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
		275					280					285			
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
		290				295					300				
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305					310					315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
				325					330					335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
			340					345					350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
		355					360					365			
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370					375					380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385					390					395					400
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
				405					410					415	
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
			420					425					430		
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Thr	Phe
		435					440					445			
Arg	Gly	Ala	Pro	Ala	Leu	Asn	Asp	Leu	Cys	Ile	Lys	Val	Asn	Asn	Trp
	450					455					460				
Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn
465					470					475					480
Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu
				485					490					495	
Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe
		500						505					510		
Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile
		515					520					525			
Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly

Li *et al.*, Degradable Clostridial Toxins

530		535		540	
Lys Lys Tyr Glu Leu Asp	Lys Tyr Thr Met Phe His Tyr Leu Arg Ala				
545		550		555	560
Gln Glu Phe Glu His Gly	Lys Ser Arg Ile Ala Leu Thr Asn Ser Val				
	565		570		575
Asn Glu Ala Leu Leu Asn	Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser				
	580		585		590
Asp Tyr Val Lys Lys Val	Asn Lys Ala Thr Glu Ala Ala Met Phe Leu				
	595		600		605
Gly Trp Val Glu Gln Leu	Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu				
	610		615		620
Val Ser Thr Thr Asp Lys	Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr				
625		630		635	640
Ile Gly Pro Ala Leu Asn	Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe				
	645		650		655
Val Gly Ala Leu Ile Phe	Ser Gly Ala Val Ile Leu Leu Glu Phe Ile				
	660		665		670
Pro Glu Ile Ala Ile Pro	Val Leu Gly Thr Phe Ala Leu Val Ser Tyr				
	675		680		685
Ile Ala Asn Lys Val Leu	Thr Val Gln Thr Ile Asp Asn Ala Leu Ser				
	690		695		700
Lys Arg Asn Glu Lys Trp	Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn				
705		710		715	720
Trp Leu Ala Lys Val Asn	Thr Gln Ile Asp Leu Ile Arg Lys Lys Met				
	725		730		735
Lys Glu Ala Leu Glu Asn	Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn				
	740		745		750
Tyr Gln Tyr Asn Gln Tyr	Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe				
	755		760		765
Asn Ile Asp Asp Leu Ser	Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala				
	770		775		780
Met Ile Asn Ile Asn Lys	Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu				
785		790		795	800
Met Asn Ser Met Ile Pro	Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp				
	805		810		815
Ala Ser Leu Lys Asp Ala	Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly				
	820		825		830
Thr Leu Ile Gly Gln Val	Asp Arg Leu Lys Asp Lys Val Asn Asn Thr				
	835		840		845
Leu Ser Thr Asp Ile Pro	Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln				
	850		855		860
Arg Leu Leu Ser Thr Phe	Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr				
865		870		875	880
Ser Ile Leu Asn Leu Arg	Tyr Glu Ser Asn His Leu Ile Asp Leu Ser				
	885		890		895
Arg Tyr Ala Ser Lys Ile	Asn Ile Gly Ser Lys Val Asn Phe Asp Pro				
	900		905		910
Ile Asp Lys Asn Gln Ile	Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile				
	915		920		925
Glu Val Ile Leu Lys Asn	Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn				
	930		935		940
Phe Ser Thr Ser Phe Trp	Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile				
945		950		955	960
Ser Leu Asn Asn Glu Tyr	Thr Ile Ile Asn Cys Met Glu Asn Asn Ser				
	965		970		975
Gly Trp Lys Val Ser Leu	Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln				
	980		985		990
Asp Thr Gln Glu Ile Lys	Gln Arg Val Val Phe Lys Tyr Ser Gln Met				
	995		1000		1005

Li et al., Degradable Clostridial Toxins

Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp Ile Phe Val Thr Ile Thr
 1010 1015 1020
 Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile
 1025 1030 1035 1040
 Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn
 1045 1050 1055
 Ile Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp
 1060 1065 1070
 Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile
 1075 1080 1085
 Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe
 1090 1095 1100
 Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu
 1105 1110 1115 1120
 Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly
 1125 1130 1135
 Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile
 1140 1145 1150
 Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys
 1155 1160 1165
 Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val
 1170 1175 1180
 Tyr Ile Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn
 1185 1190 1195 1200
 Ala Ser Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro
 1205 1210 1215
 Asp Val Gly Asn Leu Ser Gln Val Val Met Lys Ser Lys Asn Asp
 1220 1225 1230
 Gln Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly
 1235 1240 1245
 Asn Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys
 1250 1255 1260
 Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg
 1265 1270 1275 1280
 Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly
 1285 1290 1295
 Glu Arg Pro Leu
 1300

<210> 99

<211> 1306

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(1306)

<223> BoNT/A-TD-PAR4-Thrombin

<400> 99

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1 5 10 15
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
 20 25 30
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
 35 40 45
 Asp Thr Phe Thr Asn Pro Glu Gly Asp Leu Asn Pro Pro Pro Glu
 50 55 60

Li et al., Degradable Clostridial Toxins

Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser	Thr
65					70					75					80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
		115					120					125			
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130					135					140				
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150					155					160
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
				165					170					175	
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
			180					185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
		195					200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215					220				
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225					230					235					240
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
			245						250					255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
			260					265					270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
		275					280					285			
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
	290					295					300				
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305					310					315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
				325					330					335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
			340					345					350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
		355					360					365			
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370					375					380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385					390					395					400
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
				405					410					415	
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
			420					425					430		
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Pro	Arg	Gly	Tyr	Pro	Gly
		435					440					445			
Gln	Val	Cys	Ala	Asn	Asp	Ser	Asp	Thr	Leu	Ala	Leu	Asn	Asp	Leu	Cys
	450					455					460				
Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn
465					470					475					480
Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn
				485					490					495	
Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr
		500					505						510		
Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu
		515					520					525			
Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile

Li *et al.*, Degradable Clostridial Toxins

530						535						540					
Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met		
545					550					555					560		
Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile		
				565					570						575		
Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val		
			580					585						590			
Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr		
		595				600						605					
Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe		
610					615						620						
Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile		
625					630					635					640		
Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met		
				645					650					655			
Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val		
			660					665					670				
Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr		
		675					680					685					
Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr		
690					695						700						
Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr		
705					710					715					720		
Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp		
			725						730					735			
Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala		
		740						745					750				
Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu		
		755					760					765					
Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn		
770					775						780						
Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln		
785					790					795					800		
Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys		
				805					810					815			
Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr		
			820					825					830				
Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys		
		835					840					845					
Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser		
850					855						860						
Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile		
865					870					875					880		
Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn		
				885					890					895			
His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser		
		900						905					910				
Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn		
		915					920					925					
Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr		
930					935						940						
Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro		
945					950					955					960		
Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn		
			965						970					975			
Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu		
			980						985					990			
Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val		
995							1000					1005					

Li et al., Degradable Clostridial Toxins

Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr Ile Asn Arg Trp
 1010 1015 1020
 Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr
 1025 1030 1035 1040
 Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn
 1045 1050 1055
 Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp Gly Cys Arg Asp
 1060 1065 1070
 Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu
 1075 1080 1085
 Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser
 1090 1095 1100
 Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro
 1105 1110 1115 1120
 Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn
 1125 1130 1135
 Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser
 1140 1145 1150
 Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr
 1155 1160 1165
 Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val
 1170 1175 1180
 Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val Lys Asn Lys Glu
 1185 1190 1195 1200
 Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val Glu Lys Ile Leu
 1205 1210 1215
 Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser Gln Val Val Val
 1220 1225 1230
 Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys Cys Lys Met Asn
 1235 1240 1245
 Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile Gly Phe His Gln
 1250 1255 1260
 Phe Asn Asn Ile Ala Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln
 1265 1270 1275 1280
 Ile Glu Arg Ser Ser Arg Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro
 1285 1290 1295
 Val Asp Asp Gly Trp Gly Glu Arg Pro Leu
 1300 1305

<210> 100

<211> 1300

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)...(1300)

<223> BoNT/A-TD-PAR4-Xa

<400> 100

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1 5 10 15
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
 20 25 30
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
 35 40 45
 Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu

Li *et al.*, Degradable Clostridial Toxins

50	55	60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr		
65	70	75
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu		80
	85	90
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val		95
	100	105
Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys		110
	115	120
Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr		125
	130	135
Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile		140
145	150	155
Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr		160
	165	170
Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe		175
	180	185
Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu		190
	195	200
Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu		205
	210	215
Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn		220
225	230	235
Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu		240
	245	250
Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys		255
	260	265
Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn		270
	275	280
Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val		285
	290	295
Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys		300
305	310	315
Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu		320
	325	330
Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp		335
	340	345
Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn		350
	355	360
Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr		365
	370	375
Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn		380
385	390	395
Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu		400
	405	410
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg		415
	420	425
Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Ile Glu Gly Arg Gly Tyr		430
	435	440
Pro Gly Gln Val Ala Leu Asn Asp Leu Cys Ile Lys Val Asn Asn Trp		445
	450	455
Asp Leu Phe Phe Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn		460
465	470	475
Lys Gly Glu Glu Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu		480
	485	490
Asn Ile Ser Leu Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe		495
	500	505
Asp Asn Glu Pro Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile		510
	515	520
		525

Li et al., Degradable Clostridial Toxins

Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly
530						535					540				
Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu	Arg	Ala
545					550					555					560
Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	Ser	Val
				565					570					575	
Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe	Ser	Ser
				580				585					590		
Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe	Leu
		595					600					605			
Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser	Glu
610						615					620				
Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro	Tyr
625					630					635					640
Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	Asp	Phe
				645					650					655	
Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	Phe	Ile
			660					665					670		
Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	Ser	Tyr
							680					685			
Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	Leu	Ser
690						695					700				
Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	Thr	Asn
705					710					715					720
Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	Lys	Met
				725					730					735	
Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	Ile	Asn
			740					745					750		
Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe
		755					760					765			
Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala
770						775					780				
Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu
785					790					795					800
Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp
				805					810					815	
Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly
			820					825					830		
Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	Asp	Lys	Val	Asn	Asn	Thr
		835					840					845			
Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	Lys	Tyr	Val	Asp	Asn	Gln
850						855					860				
Arg	Leu	Leu	Ser	Thr	Phe	Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	Asn	Thr
865					870					875					880
Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	Leu	Ser
			885						890					895	
Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	Asp	Pro
		900						905					910		
Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu	Ser	Ser	Lys	Ile
		915					920					925			
Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser	Met	Tyr	Glu	Asn
930						935					940				
Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Ser	Ile
945					950					955					960
Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met	Glu	Asn	Asn	Ser
			965						970					975	
Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile	Trp	Thr	Leu	Gln
		980						985					990		
Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys	Tyr	Ser	Gln	Met

Li et al., Degradable Clostridial Toxins

995	1000	1005
Ile Asn Ile Ser Asp Tyr	Ile Asn Arg Trp Ile	Phe Val Thr Ile Thr
1010	1015	1020
Asn Asn Arg Leu Asn Asn Ser Lys Ile Tyr	Ile Asn Gly Arg Leu Ile	
1025	1030	1035
Asp Gln Lys Pro Ile Ser Asn Leu Gly Asn Ile His Ala Ser Asn Asn		1040
1045	1050	1055
Ile Met Phe Lys Leu Asp Gly Cys Arg Asp Thr His Arg Tyr Ile Trp		
1060	1065	1070
Ile Lys Tyr Phe Asn Leu Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile		
1075	1080	1085
Lys Asp Leu Tyr Asp Asn Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe		
1090	1095	1100
Trp Gly Asp Tyr Leu Gln Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu		
1105	1110	1115
Tyr Asp Pro Asn Lys Tyr Val Asp Val Asn Asn Val Gly Ile Arg Gly		
1125	1130	1135
Tyr Met Tyr Leu Lys Gly Pro Arg Gly Ser Val Met Thr Thr Asn Ile		
1140	1145	1150
Tyr Leu Asn Ser Ser Leu Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys		
1155	1160	1165
Tyr Ala Ser Gly Asn Lys Asp Asn Ile Val Arg Asn Asn Asp Arg Val		
1170	1175	1180
Tyr Ile Asn Val Val Val Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn		
1185	1190	1195
Ala Ser Gln Ala Gly Val Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro		
1205	1210	1215
Asp Val Gly Asn Leu Ser Gln Val Val Val Met Lys Ser Lys Asn Asp		
1220	1225	1230
Gln Gly Ile Thr Asn Lys Cys Lys Met Asn Leu Gln Asp Asn Asn Gly		
1235	1240	1245
Asn Asp Ile Gly Phe Ile Gly Phe His Gln Phe Asn Asn Ile Ala Lys		
1250	1255	1260
Leu Val Ala Ser Asn Trp Tyr Asn Arg Gln Ile Glu Arg Ser Ser Arg		
1265	1270	1275
Thr Leu Gly Cys Ser Trp Glu Phe Ile Pro Val Asp Asp Gly Trp Gly		
1285	1290	1295
Glu Arg Pro Leu		
1300		

<210> 101

<211> 1329

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1) ... (1329)

<223> BoNT/A-BD-PAR1-Thrombin

<400> 101

Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
1 5 10 15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
20 25 30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg

35

40

45

Li et al., Degradable Clostridial Toxins

Asp	Thr	Phe	Thr	Asn	Pro	Glu	Glu	Gly	Asp	Leu	Asn	Pro	Pro	Pro	Glu
50						55				60					
Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser	Thr
65					70					75					80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
	115						120					125			
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130					135				140					
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150					155					160
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
				165					170					175	
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
			180					185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
			195				200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215					220				
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225					230					235					240
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
				245					250					255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
			260					265					270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
			275				280					285			
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
			290				295				300				
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305					310					315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
				325					330					335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
			340					345					350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
			355				360					365			
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370					375					380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385					390					395					400
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
				405					410					415	
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
			420					425					430		
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Pro	Arg	Ser	Phe	Leu	Leu
			435				440					445			
Arg	Asn	Pro	Asn	Asp	Lys	Tyr	Glu	Pro	Phe	Ala	Leu	Asn	Asp	Leu	Phe
						455				460					
Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg
465					470					475					480
Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile
				485					490					495	
Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile
				500				505					510		
Gln	Leu	Phe	Asn	Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn

Li *et al.*, Degradable Clostridial Toxins

515						520					525				
Ala	Ile	Val	Tyr	Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp
530						535					540				
Ile	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr
545						550					555				
Thr	Ile	Ile	Asn	Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu
565						570					575				
Asn	Tyr	Gly	Glu	Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys
580						585					590				
Gln	Arg	Val	Val	Phe	Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr
595						600					605				
Ile	Asn	Arg	Trp	Ile	Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn
610						615					620				
Ser	Lys	Ile	Tyr	Ile	Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser
625						630					635				
Asn	Leu	Gly	Asn	Ile	His	Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp
645						650					655				
Gly	Cys	Arg	Asp	Thr	His	Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu
660						665					670				
Phe	Asp	Lys	Glu	Leu	Asn	Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn
675						680					685				
Gln	Ser	Asn	Ser	Gly	Ile	Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln
690						695					700				
Tyr	Asp	Lys	Pro	Tyr	Tyr	Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr
705						710					715				
Val	Asp	Val	Asn	Asn	Val	Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly
725						730					735				
Pro	Arg	Gly	Ser	Val	Met	Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu
740						745					750				
Tyr	Arg	Gly	Thr	Lys	Phe	Ile	Ile	Lys	Lys	Tyr	Ala	Ser	Gly	Asn	Lys
755						760					765				
Asp	Asn	Ile	Val	Arg	Asn	Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val
770						775					780				
Lys	Asn	Lys	Glu	Tyr	Arg	Leu	Ala	Thr	Asn	Ala	Ser	Gln	Ala	Gly	Val
785						790					795				
Glu	Lys	Ile	Leu	Ser	Ala	Leu	Glu	Ile	Pro	Asp	Val	Gly	Asn	Leu	Ser
805						810					815				
Gln	Val	Val	Val	Met	Lys	Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys
820						825					830				
Cys	Lys	Met	Asn	Leu	Gln	Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile
835						840					845				
Gly	Phe	His	Gln	Phe	Asn	Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp
850						855					860				
Tyr	Asn	Arg	Gln	Ile	Glu	Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp
865						870					875				
Glu	Phe	Ile	Pro	Val	Asp	Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu
885						890					895				
Ala	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser
900						905					910				
Ala	Leu	Val	Leu	Gln	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe
915						920					925				
Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu
930						935					940				
Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu
945						950					955				
Asp	Leu	Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro
965						970					975				
Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu
980						985					990				

Li *et al.*, Degradable Clostridial Toxins

Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu
 995 1000 1005
 Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu
 1010 1015 1020
 His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu
 1025 1030 1035 1040
 Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys
 1045 1050 1055
 Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu
 1060 1065 1070
 Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr
 1075 1080 1085
 Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala
 1090 1095 1100
 Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu
 1105 1110 1115 1120
 Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala
 1125 1130 1135
 Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys
 1140 1145 1150
 Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
 1155 1160 1165
 Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys
 1170 1175 1180
 Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu
 1185 1190 1195 1200
 Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn
 1205 1210 1215
 Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp
 1220 1225 1230
 Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile
 1235 1240 1245
 Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met
 1250 1255 1260
 Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys
 1265 1270 1275 1280
 Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly
 1285 1290 1295
 Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp
 1300 1305 1310
 Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser
 1315 1320 1325
 Thr

<210> 102
 <211> 1323
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> PEPTIDE
 <222> (1)...(1323)
 <223> BoNT/A-BD-PAR1-Xa

<400> 102
 Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1 5 10 15

Li et al., Degradable Clostridial Toxins

Val	Asp	Ile	Ala	Tyr	Ile	Lys	Ile	Pro	Asn	Ala	Gly	Gln	Met	Gln	Pro
			20					25					30		
Val	Lys	Ala	Phe	Lys	Ile	His	Asn	Lys	Ile	Trp	Val	Ile	Pro	Glu	Arg
		35					40					45			
Asp	Thr	Phe	Thr	Asn	Pro	Glu	Glu	Gly	Asp	Leu	Asn	Pro	Pro	Pro	Glu
	50					55					60				
Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser	Thr
65					70					75					80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
		115					120					125			
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130					135					140				
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150					155					160
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
				165					170					175	
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
			180					185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
	195						200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215					220				
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225					230					235					240
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
			245						250					255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
			260					265					270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
		275					280					285			
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
	290					295					300				
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305					310					315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
			325						330					335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
			340					345					350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
		355					360					365			
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370					375					380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385					390					395					400
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
				405					410					415	
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
			420					425					430		
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Ser	Phe
	435						440					445			
Leu	Leu	Arg	Asn	Ala	Leu	Asn	Asp	Leu	Phe	Thr	Glu	Tyr	Ile	Lys	Asn
	450					455					460				
Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu
465					470					475					480
Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val

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																485			490			495		
Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu									
			500					505					510											
Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser									
		515					520					525												
Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr									
		530				535					540													
Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met									
545					550				555						560									
Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile									
				565					570					575										
Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys									
			580					585					590											
Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile	Phe									
		595					600					605												
Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	Ile	Asn									
		610				615					620													
Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	Ile	His									
625					630					635					640									
Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	Asp	Thr	His									
				645					650					655										
Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu	Phe	Asp	Lys	Glu	Leu	Asn									
			660					665					670											
Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn	Gln	Ser	Asn	Ser	Gly	Ile									
		675					680					685												
Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln	Tyr	Asp	Lys	Pro	Tyr	Tyr									
		690				695					700													
Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr	Val	Asp	Val	Asn	Asn	Val									
705					710					715					720									
Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly	Pro	Arg	Gly	Ser	Val	Met									
				725					730					735										
Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu	Tyr	Arg	Gly	Thr	Lys	Phe									
			740					745					750											
Ile	Ile	Lys	Lys	Tyr	Ala	Ser	Gly	Asn	Lys	Asp	Asn	Ile	Val	Arg	Asn									
		755					760					765												
Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val	Lys	Asn	Lys	Glu	Tyr	Arg									
		770				775					780													
Leu	Ala	Thr	Asn	Ala	Ser	Gln	Ala	Gly	Val	Glu	Lys	Ile	Leu	Ser	Ala									
785					790				795					800										

Li et al., Degradable Clostridial Toxins

Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu
				965					970					975	
Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile
			980					985					990		
Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met
		995					1000					1005			
Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile
	1010					1015					1020				
Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val
1025					1030					1035					1040
Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr
				1045					1050					1055	
Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe
			1060					1065					1070		
Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile
		1075					1080					1085			
Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met
	1090					1095					1100				
Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val
1105					1110					1115					1120
Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr
				1125					1130					1135	
Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr
			1140					1145					1150		
Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr
		1155					1160					1165			
Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp
	1170					1175					1180				
Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala
1185					1190					1195					1200
Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu
				1205					1210					1215	
Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn
			1220					1225					1230		
Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln
		1235					1240					1245			
Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys
	1250					1255					1260				
Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr
1265					1270					1275					1280
Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys
			1285												

<210> 103

<211> 1329

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1) ... (1329)

<223> BoNT/A-BD-PAR2-Trypsin

<400> 103

Li et al., Degradable Clostridial Toxins

Met 1	Pro	Phe	Val	Asn 5	Lys	Gln	Phe	Asn 10	Tyr	Lys	Asp	Pro	Val	Asn 15	Gly
Val	Asp	Ile	Ala	Tyr 20	Ile	Lys	Ile	Pro 25	Asn	Ala	Gly	Gln	Met 30	Gln	Pro
Val	Lys	Ala	Phe	Lys	Ile	His	Asn 40	Lys	Ile	Trp	Val	Ile	Pro	Glu	Arg
Asp	Thr 50	Phe	Thr	Asn	Pro	Glu 55	Glu	Gly	Asp	Leu	Asn 60	Pro	Pro	Pro	Glu
Ala 65	Lys	Gln	Val	Pro 70	Val	Ser	Tyr	Tyr	Asp 75	Ser	Thr	Tyr	Leu	Ser 80	Thr
Asp	Asn	Glu	Lys	Asp 85	Asn	Tyr	Leu	Lys	Gly 90	Val	Thr	Lys	Leu	Phe 95	Glu
Arg	Ile	Tyr	Ser 100	Thr	Asp	Leu	Gly	Arg 105	Met	Leu	Leu	Thr	Ser 110	Ile	Val
Arg	Gly	Ile 115	Pro	Phe	Trp	Gly	Gly 120	Ser	Thr	Ile	Asp 125	Thr	Glu	Leu	Lys
Val 130	Ile	Asp	Thr	Asn	Cys 135	Ile	Asn	Val	Ile	Gln	Pro 140	Asp	Gly	Ser	Tyr
Arg 145	Ser	Glu	Glu	Leu 150	Asn	Leu	Val	Ile	Ile	Gly 155	Pro	Ser	Ala	Asp 160	Ile
Ile	Gln	Phe	Glu	Cys 165	Lys	Ser	Phe	Gly	His 170	Glu	Val	Leu	Asn	Leu 175	Thr
Arg	Asn	Gly	Tyr 180	Gly	Ser	Thr	Gln	Tyr 185	Ile	Arg	Phe	Ser	Pro 190	Asp	Phe
Thr	Phe	Gly 195	Phe	Glu	Glu	Ser	Leu 200	Glu	Val	Asp	Thr 205	Asn	Pro	Leu	Leu
Gly 210	Ala	Gly	Lys	Phe	Ala 215	Thr	Asp	Pro	Ala	Val	Thr 220	Leu	Ala	His	Glu
Leu 225	Ile	His	Ala	Gly 230	His	Arg	Leu	Tyr	Gly	Ile 235	Ala	Ile	Asn	Pro 240	Asn
Arg	Val	Phe	Lys	Val 245	Asn	Thr	Asn	Ala	Tyr 250	Tyr	Glu	Met	Ser	Gly 255	Leu
Glu	Val	Ser	Phe 260	Glu	Glu	Leu	Arg	Thr 265	Phe	Gly	Gly	His 270	Asp	Ala	Lys
Phe	Ile	Asp 275	Ser	Leu	Gln	Glu	Asn 280	Glu	Phe	Arg	Leu 285	Tyr	Tyr	Tyr	Asn
Lys	Phe 290	Lys	Asp	Ile	Ala 295	Ser	Thr	Leu	Asn	Lys	Ala 300	Lys	Ser	Ile	Val
Gly 305	Thr	Thr	Ala	Ser 310	Leu	Gln	Tyr	Met	Lys	Asn 315	Val	Phe	Lys	Glu	Lys
Tyr	Leu	Leu	Ser	Glu 325	Asp	Thr	Ser	Gly	Lys 330	Phe	Ser	Val	Asp	Lys 335	Leu
Lys	Phe	Asp 340	Lys	Leu	Tyr	Lys	Met 345	Leu	Thr	Glu	Ile 350	Tyr	Thr	Glu	Asp
Asn	Phe 355	Val	Lys	Phe	Phe	Lys	Val 360	Leu	Asn	Arg	Lys 365	Thr	Tyr	Leu	Asn
Phe	Asp 370	Lys	Ala	Val	Phe	Lys 375	Ile	Asn	Ile	Val	Pro 380	Lys	Val	Asn	Tyr
Thr 385	Ile	Tyr	Asp	Gly 390	Phe	Asn	Leu	Arg	Asn 395	Thr	Asn	Leu	Ala	Ala	Asn
Phe	Asn	Gly	Gln	Asn 405	Thr	Glu	Ile	Asn 410	Asn	Met	Asn	Phe	Thr	Lys 415	Leu
Lys	Asn	Phe 420	Thr	Gly	Leu	Phe	Glu	Phe 425	Tyr	Lys	Leu	Leu	Cys	Val	Arg
Gly	Ile 435	Ile	Thr	Ser	Lys	Thr	Lys 440	Ser	Leu	Gly	Arg 445	Ser	Leu	Ile	Gly
Lys	Val 450	Asp	Gly	Thr	Ser	His 455	Val	Thr	Gly	Ala 460	Leu	Asn	Asp	Leu	Phe
Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg

Li et al., Degradable Clostridial Toxins

465					470					475					480
Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile
				485						490				495	
Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile
			500					505					510		
Gln	Leu	Phe	Asn	Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn
		515					520					525			
Ala	Ile	Val	Tyr	Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp
	530					535					540				
Ile	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr
545					550					555				560	
Thr	Ile	Ile	Asn	Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu
			565					570					575		
Asn	Tyr	Gly	Glu	Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys
		580					585					590			
Gln	Arg	Val	Val	Phe	Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr
	595					600					605				
Ile	Asn	Arg	Trp	Ile	Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn
	610				615					620					
Ser	Lys	Ile	Tyr	Ile	Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser
625				630					635					640	
Asn	Leu	Gly	Asn	Ile	His	Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp
			645					650					655		
Gly	Cys	Arg	Asp	Thr	His	Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu
		660					665					670			
Phe	Asp	Lys	Glu	Leu	Asn	Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn
	675					680					685				
Gln	Ser	Asn	Ser	Gly	Ile	Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln
	690				695					700					
Tyr	Asp	Lys	Pro	Tyr	Tyr	Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr
705				710					715					720	
Val	Asp	Val	Asn	Asn	Val	Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly
			725					730					735		
Pro	Arg	Gly	Ser	Val	Met	Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu
		740					745					750			
Tyr	Arg	Gly	Thr	Lys	Phe	Ile	Ile	Lys	Lys	Tyr	Ala	Ser	Gly	Asn	Lys
	755					760				765					
Asp	Asn	Ile	Val	Arg	Asn	Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val
	770				775					780					
Lys	Asn	Lys	Glu	Tyr	Arg	Leu	Ala	Thr	Asn	Ala	Ser	Gln	Ala	Gly	Val
785				790					795					800	
Glu	Lys	Ile	Leu	Ser	Ala	Leu	Glu	Ile	Pro	Asp	Val	Gly	Asn	Leu	Ser
			805					810					815		
Gln	Val	Val	Val	Met	Lys	Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys
		820					825					830			
Cys	Lys	Met	Asn	Leu	Gln	Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile
	835					840					845				
Gly	Phe	His	Gln	Phe	Asn	Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp
	850				855						860				
Tyr	Asn	Arg	Gln	Ile	Glu	Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp
865				870					875					880	
Glu	Phe	Ile	Pro	Val	Asp	Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu
			885					890					895		
Ala	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser
		900					905					910			
Ala	Leu	Val	Leu	Gln	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe
	915					920					925				
Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu
	930				935						940				

Li et al., Degradable Clostridial Toxins

Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu
 945 950 955 960
 Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro
 965 970 975
 Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu
 980 985 990
 Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu
 995 1000 1005
 Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu
 1010 1015 1020
 His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu
 1025 1030 1035 1040
 Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys
 1045 1050 1055
 Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu
 1060 1065 1070
 Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr
 1075 1080 1085
 Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala
 1090 1095 1100
 Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu
 1105 1110 1115 1120
 Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala
 1125 1130 1135
 Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys
 1140 1145 1150
 Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
 1155 1160 1165
 Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys
 1170 1175 1180
 Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu
 1185 1190 1195 1200
 Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn
 1205 1210 1215
 Gln Tyr Thr Glu Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp
 1220 1225 1230
 Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile
 1235 1240 1245
 Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met
 1250 1255 1260
 Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys
 1265 1270 1275 1280
 Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly
 1285 1290 1295
 Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp
 1300 1305 1310
 Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser
 1315 1320 1325
 Thr

<210> 104

<211> 1323

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

Li et al., Degradable Clostridial Toxins

<222> (1) ... (1323)

<223> BoNT/A-BD-PAR2-Xa

<400> 104

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Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1          5          10          15
Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
 20          25          30
Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
 35          40          45
Asp Thr Phe Thr Asn Pro Glu Gly Asp Leu Asn Pro Pro Pro Glu
 50          55          60
Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
 65          70          75          80
Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
 85          90          95
Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
 100         105         110
Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
 115         120         125
Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
 130         135         140
Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
 145         150         155         160
Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
 165         170         175
Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
 180         185         190
Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
 195         200         205
Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
 210         215         220
Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
 225         230         235         240
Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
 245         250         255
Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
 260         265         270
Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
 275         280         285
Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
 290         295         300
Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
 305         310         315         320
Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
 325         330         335
Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
 340         345         350
Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
 355         360         365
Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
 370         375         380
Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn
 385         390         395         400
Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu
 405         410         415
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg
 420         425         430
Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Ile Glu Gly Arg Ser Leu

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Li *et al.*, Degradable Clostridial Toxins

		435					440					445			
Ile	Gly	Lys	Val	Ala	Leu	Asn	Asp	Leu	Phe	Thr	Glu	Tyr	Ile	Lys	Asn
	450					455					460				
Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu
465					470					475					480
Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val
				485					490					495	
Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu
		500					505						510		
Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser
	515					520						525			
Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr
	530					535					540				
Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met
545					550					555					560
Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile
				565					570					575	
Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys
			580				585						590		
Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile	Phe
	595					600					605				
Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	Ile	Asn
	610					615					620				
Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	Ile	His
625					630					635					640
Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	Asp	Thr	His
				645					650					655	
Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu	Phe	Asp	Lys	Glu	Leu	Asn
			660					665					670		
Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn	Gln	Ser	Asn	Ser	Gly	Ile
			675				680					685			
Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln	Tyr	Asp	Lys	Pro	Tyr	Tyr
	690					695					700				
Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr	Val	Asp	Val	Asn	Asn	Val
705					710					715					720
Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly	Pro	Arg	Gly	Ser	Val	Met
				725					730					735	
Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu	Tyr	Arg	Gly	Thr	Lys	Phe
			740					745					750		
Ile	Ile	Lys	Tyr	Ala	Ser	Gly	Asn	Lys	Asp	Asn	Ile	Val	Arg	Asn	
		755				760					765				
Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val	Lys	Asn	Lys	Glu	Tyr	Arg
						775					780				
Leu	Ala	Thr	Asn	Ala	Ser	Gln	Ala	Gly	Val	Glu	Lys	Ile	Leu	Ser	Ala
785					790					795					800
Leu	Glu	Ile	Pro	Asp	Val	Gly	Asn	Leu	Ser	Gln	Val	Val	Val	Met	Lys
				805					810					815	
Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys	Cys	Lys	Met	Asn	Leu	Gln
				820				825					830		
Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile	Gly	Phe	His	Gln	Phe	Asn
			835				840					845			
Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp	Tyr	Asn	Arg	Gln	Ile	Glu
	850					855					860				
Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp	Glu	Phe	Ile	Pro	Val	Asp
865					870					875					880
Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu	Ala	Gly	Gly	Gly	Gly	Ser
				885					890					895	
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ala	Leu	Val	Leu	Gln	Cys
			900					905					910		

Li et al., Degradable Clostridial Toxins

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Ile Lys Val Asn Asn Trp Asp Leu Phe Phe Ser Pro Ser Glu Asp Asn
   915                               920               925
Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu Ile Thr Ser Asp Thr Asn
   930                               935               940
Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu Asp Leu Ile Gln Gln Tyr
  945                               950               955               960
Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro Glu Asn Ile Ser Ile Glu
                               965               970               975
Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu Glu Leu Met Pro Asn Ile
                               980               985               990
Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu Leu Asp Lys Tyr Thr Met
   995                               1000               1005
Phe His Tyr Leu Arg Ala Gln Glu Phe Glu His Gly Lys Ser Arg Ile
  1010                               1015               1020
Ala Leu Thr Asn Ser Val Asn Glu Ala Leu Leu Asn Pro Ser Arg Val
 1025                               1030               1035               1040
Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys Lys Val Asn Lys Ala Thr
                               1045               1050               1055
Glu Ala Ala Met Phe Leu Gly Trp Val Glu Gln Leu Val Tyr Asp Phe
                               1060               1065               1070
Thr Asp Glu Thr Ser Glu Val Ser Thr Thr Asp Lys Ile Ala Asp Ile
                               1075               1080               1085
Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly Asn Met
 1090                               1095               1100
Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu Ile Phe Ser Gly Ala Val
 1105                               1110               1115               1120
Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala Ile Pro Val Leu Gly Thr
                               1125               1130               1135
Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys Val Leu Thr Val Gln Thr
                               1140               1145               1150
Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu Lys Trp Asp Glu Val Tyr
                               1155               1160               1165
Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys Val Asn Thr Gln Ile Asp
 1170                               1175               1180
Leu Ile Arg Lys Lys Met Lys Glu Ala Leu Glu Asn Gln Ala Glu Ala
 1185                               1190               1195               1200
Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn Gln Tyr Thr Glu Glu Glu
                               1205               1210               1215
Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp Leu Ser Ser Lys Leu Asn
                               1220               1225               1230
Glu Ser Ile Asn Lys Ala Met Ile Asn Ile Asn Lys Phe Leu Asn Gln
 1235                               1240               1245
Cys Ser Val Ser Tyr Leu Met Asn Ser Met Ile Pro Tyr Gly Val Lys
 1250                               1255               1260
Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys Asp Ala Leu Leu Lys Tyr
 1265                               1270               1275               1280
Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly Gln Val Asp Arg Leu Lys
                               1285               1290               1295
Asp Lys Val Asn Asn Thr Leu Ser Thr Asp Ile Pro Phe Gln Leu Ser
 1300                               1305               1310
Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser Thr
 1315                               1320

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<210> 105

<211> 1329

<212> PRT

<213> Artificial Sequence

Li et al., Degradable Clostridial Toxins

<220>

<221> PEPTIDE

<222> (1)...(1329)

<223> BoNT/A-BD-PAR3-Thrombin

<400> 105

Met	Pro	Phe	Val	Asn	Lys	Gln	Phe	Asn	Tyr	Lys	Asp	Pro	Val	Asn	Gly
1				5				10						15	
Val	Asp	Ile	Ala	Tyr	Ile	Lys	Ile	Pro	Asn	Ala	Gly	Gln	Met	Gln	Pro
			20					25					30		
Val	Lys	Ala	Phe	Lys	Ile	His	Asn	Lys	Ile	Trp	Val	Ile	Pro	Glu	Arg
		35					40					45			
Asp	Thr	Phe	Thr	Asn	Pro	Glu	Glu	Gly	Asp	Leu	Asn	Pro	Pro	Pro	Glu
	50					55					60				
Ala	Lys	Gln	Val	Pro	Val	Ser	Tyr	Tyr	Asp	Ser	Thr	Tyr	Leu	Ser	Thr
65					70					75					80
Asp	Asn	Glu	Lys	Asp	Asn	Tyr	Leu	Lys	Gly	Val	Thr	Lys	Leu	Phe	Glu
				85					90					95	
Arg	Ile	Tyr	Ser	Thr	Asp	Leu	Gly	Arg	Met	Leu	Leu	Thr	Ser	Ile	Val
			100					105					110		
Arg	Gly	Ile	Pro	Phe	Trp	Gly	Gly	Ser	Thr	Ile	Asp	Thr	Glu	Leu	Lys
	115					120						125			
Val	Ile	Asp	Thr	Asn	Cys	Ile	Asn	Val	Ile	Gln	Pro	Asp	Gly	Ser	Tyr
	130				135						140				
Arg	Ser	Glu	Glu	Leu	Asn	Leu	Val	Ile	Ile	Gly	Pro	Ser	Ala	Asp	Ile
145					150					155					160
Ile	Gln	Phe	Glu	Cys	Lys	Ser	Phe	Gly	His	Glu	Val	Leu	Asn	Leu	Thr
				165				170						175	
Arg	Asn	Gly	Tyr	Gly	Ser	Thr	Gln	Tyr	Ile	Arg	Phe	Ser	Pro	Asp	Phe
			180					185					190		
Thr	Phe	Gly	Phe	Glu	Glu	Ser	Leu	Glu	Val	Asp	Thr	Asn	Pro	Leu	Leu
		195					200					205			
Gly	Ala	Gly	Lys	Phe	Ala	Thr	Asp	Pro	Ala	Val	Thr	Leu	Ala	His	Glu
	210					215					220				
Leu	Ile	His	Ala	Gly	His	Arg	Leu	Tyr	Gly	Ile	Ala	Ile	Asn	Pro	Asn
225					230					235					240
Arg	Val	Phe	Lys	Val	Asn	Thr	Asn	Ala	Tyr	Tyr	Glu	Met	Ser	Gly	Leu
			245					250						255	
Glu	Val	Ser	Phe	Glu	Glu	Leu	Arg	Thr	Phe	Gly	Gly	His	Asp	Ala	Lys
		260						265					270		
Phe	Ile	Asp	Ser	Leu	Gln	Glu	Asn	Glu	Phe	Arg	Leu	Tyr	Tyr	Tyr	Asn
	275						280					285			
Lys	Phe	Lys	Asp	Ile	Ala	Ser	Thr	Leu	Asn	Lys	Ala	Lys	Ser	Ile	Val
	290					295					300				
Gly	Thr	Thr	Ala	Ser	Leu	Gln	Tyr	Met	Lys	Asn	Val	Phe	Lys	Glu	Lys
305					310					315					320
Tyr	Leu	Leu	Ser	Glu	Asp	Thr	Ser	Gly	Lys	Phe	Ser	Val	Asp	Lys	Leu
			325					330						335	
Lys	Phe	Asp	Lys	Leu	Tyr	Lys	Met	Leu	Thr	Glu	Ile	Tyr	Thr	Glu	Asp
		340						345					350		
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
		355					360					365			
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
	370					375					380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385					390					395					400
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
			405					410						415	
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg

Li *et al.*, Degradable Clostridial Toxins

			420					425				430			
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Lys	Thr	Phe	Arg	Gly
			435					440				445			
Ala	Pro	Pro	Asn	Ser	Phe	Glu	Glu	Phe	Pro	Ala	Leu	Asn	Asp	Leu	Phe
			450					455				460			
Thr	Glu	Tyr	Ile	Lys	Asn	Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg
465						470				475					480
Tyr	Glu	Ser	Asn	His	Leu	Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile
				485					490						495
Asn	Ile	Gly	Ser	Lys	Val	Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile
			500					505						510	
Gln	Leu	Phe	Asn	Leu	Glu	Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn
			515					520				525			
Ala	Ile	Val	Tyr	Asn	Ser	Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp
			530				535					540			
Ile	Arg	Ile	Pro	Lys	Tyr	Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr
545						550				555					560
Thr	Ile	Ile	Asn	Cys	Met	Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu
				565					570						575
Asn	Tyr	Gly	Glu	Ile	Ile	Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys
			580					585							590
Gln	Arg	Val	Val	Phe	Lys	Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr
			595				600					605			
Ile	Asn	Arg	Trp	Ile	Phe	Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn
			610				615					620			
Ser	Lys	Ile	Tyr	Ile	Asn	Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser
625					630					635					640
Asn	Leu	Gly	Asn	Ile	His	Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp
				645					650						655
Gly	Cys	Arg	Asp	Thr	His	Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu
			660					665					670		
Phe	Asp	Lys	Glu	Leu	Asn	Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn
			675				680					685			
Gln	Ser	Asn	Ser	Gly	Ile	Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln
			690				695				700				
Tyr	Asp	Lys	Pro	Tyr	Tyr	Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr
705						710				715					720
Val	Asp	Val	Asn	Asn	Val	Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly
				725					730						735
Pro	Arg	Gly	Ser	Val	Met	Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu
			740					745					750		
Tyr	Arg	Gly	Thr	Lys	Phe	Ile	Ile	Lys	Lys	Tyr	Ala	Ser	Gly	Asn	Lys
			755				760					765			
Asp	Asn	Ile	Val	Arg	Asn	Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val
			770				775				780				
Lys	Asn	Lys	Glu	Tyr	Arg	Leu	Ala	Thr	Asn	Ala	Ser	Gln	Ala	Gly	Val
785						790				795					800
Glu	Lys	Ile	Leu	Ser	Ala	Leu	Glu	Ile	Pro	Asp	Val	Gly	Asn	Leu	Ser
				805					810					815	
Gln	Val	Val	Val	Met	Lys	Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys
				820				825					830		
Cys	Lys	Met	Asn	Leu	Gln	Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile
			835				840					845			
Gly	Phe	His	Gln	Phe	Asn	Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp
			850				855				860				
Tyr	Asn	Arg	Gln	Ile	Glu	Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp
865						870				875					880
Glu	Phe	Ile	Pro	Val	Asp	Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu
				885					890						895

Li et al., Degradable Clostridial Toxins

Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 900 905 910
 Ala Leu Val Leu Gln Cys Ile Lys Val Asn Asn Trp Asp Leu Phe Phe
 915 920 925
 Ser Pro Ser Glu Asp Asn Phe Thr Asn Asp Leu Asn Lys Gly Glu Glu
 930 935 940
 Ile Thr Ser Asp Thr Asn Ile Glu Ala Ala Glu Glu Asn Ile Ser Leu
 945 950 955 960
 Asp Leu Ile Gln Gln Tyr Tyr Leu Thr Phe Asn Phe Asp Asn Glu Pro
 965 970 975
 Glu Asn Ile Ser Ile Glu Asn Leu Ser Ser Asp Ile Ile Gly Gln Leu
 980 985 990
 Glu Leu Met Pro Asn Ile Glu Arg Phe Pro Asn Gly Lys Lys Tyr Glu
 995 1000 1005
 Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu
 1010 1015 1020
 His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu
 1025 1030 1035 1040
 Leu Asn Pro Ser Arg Val Tyr Thr Phe Phe Ser Ser Asp Tyr Val Lys
 1045 1050 1055
 Lys Val Asn Lys Ala Thr Glu Ala Ala Met Phe Leu Gly Trp Val Glu
 1060 1065 1070
 Gln Leu Val Tyr Asp Phe Thr Asp Glu Thr Ser Glu Val Ser Thr Thr
 1075 1080 1085
 Asp Lys Ile Ala Asp Ile Thr Ile Ile Ile Pro Tyr Ile Gly Pro Ala
 1090 1095 1100
 Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu
 1105 1110 1115 1120
 Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala
 1125 1130 1135
 Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys
 1140 1145 1150
 Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
 1155 1160 1165
 Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys
 1170 1175 1180
 Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu
 1185 1190 1195 1200
 Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn
 1205 1210 1215
 Gln Tyr Thr Glu Glu Lys Asn Asn Ile Asn Phe Asn Ile Asp Asp
 1220 1225 1230
 Leu Ser Ser Lys Leu Asn Glu Ser Ile Asn Lys Ala Met Ile Asn Ile
 1235 1240 1245
 Asn Lys Phe Leu Asn Gln Cys Ser Val Ser Tyr Leu Met Asn Ser Met
 1250 1255 1260
 Ile Pro Tyr Gly Val Lys Arg Leu Glu Asp Phe Asp Ala Ser Leu Lys
 1265 1270 1275 1280
 Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly
 1285 1290 1295
 Gln Val Asp Arg Leu Lys Asp Lys Val Asn Asn Thr Leu Ser Thr Asp
 1300 1305 1310
 Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser
 1315 1320 1325
 Thr

Li *et al.*, Degradable Clostridial Toxins

<211> 1323
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> PEPTIDE
 <222> (1)...(1323)
 <223> BoNT/A-BD-PAR3-Xa

<400> 106
 Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1 5 10 15
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
 20 25 30
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
 35 40 45
 Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
 50 55 60
 Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
 65 70 75 80
 Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
 85 90 95
 Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
 100 105 110
 Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
 115 120 125
 Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
 130 135 140
 Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
 145 150 155 160
 Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
 165 170 175
 Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
 180 185 190
 Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
 195 200 205
 Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
 210 215 220
 Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
 225 230 235 240
 Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
 245 250 255
 Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
 260 265 270
 Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
 275 280 285
 Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
 290 295 300
 Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
 305 310 315 320
 Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
 325 330 335
 Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
 340 345 350
 Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
 355 360 365
 Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
 370 375 380
 Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn

Li et al., Degradable Clostridial Toxins

385					390					395					400
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
				405					410					415	
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
			420					425					430		
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Thr	Phe
		435					440					445			
Arg	Gly	Ala	Pro	Ala	Leu	Asn	Asp	Leu	Phe	Thr	Glu	Tyr	Ile	Lys	Asn
	450					455					460				
Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu
465					470					475					480
Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val
				485				490						495	
Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu
			500					505					510		
Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser
		515					520					525			
Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr
	530					535					540				
Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met
545					550					555					560
Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile
				565				570						575	
Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys
			580					585					590		
Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile	Phe
		595					600					605			
Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	Ile	Asn
	610					615					620				
Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	Ile	His
625					630					635					640
Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	Asp	Thr	His
				645				650						655	
Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu	Phe	Asp	Lys	Glu	Leu	Asn
			660					665					670		
Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn	Gln	Ser	Asn	Ser	Gly	Ile
		675					680					685			
Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln	Tyr	Asp	Lys	Pro	Tyr	Tyr
	690					695					700				
Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr	Val	Asp	Val	Asn	Asn	Val
705					710					715					720
Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly	Pro	Arg	Gly	Ser	Val	Met
				725					730					735	
Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu	Tyr	Arg	Gly	Thr	Lys	Phe
			740					745					750		
Ile	Ile	Lys	Lys	Tyr	Ala	Ser	Gly	Asn	Lys	Asp	Asn	Ile	Val	Arg	Asn
		755					760					765			
Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val	Lys	Asn	Lys	Glu	Tyr	Arg
	770					775					780				
Leu	Ala	Thr	Asn	Ala	Ser	Gln	Ala	Gly	Val	Glu	Lys	Ile	Leu	Ser	Ala
785					790					795					800
Leu	Glu	Ile	Pro	Asp	Val	Gly	Asn	Leu	Ser	Gln	Val	Val	Val	Met	Lys
				805				810						815	
Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys	Cys	Lys	Met	Asn	Leu	Gln
			820					825					830		
Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile	Gly	Phe	His	Gln	Phe	Asn
	835						840					845			
Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp	Tyr	Asn	Arg	Gln	Ile	Glu
	850					855					860				

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Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp	Glu	Phe	Ile	Pro	Val	Asp	865	870	875	880
Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu	Ala	Gly	Gly	Gly	Gly	Ser	885	890		895
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ala	Leu	Val	Leu	Gln	Cys	900	905		910
Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn	915	920		925
Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn	930	935		940
Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr	945	950		955
Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu	965	970		975
Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile	980	985		990
Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	Leu	Asp	Lys	Tyr	Thr	Met	995	1000		1005
Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile	1010	1015		1020
Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val	1025	1030		1035
Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	Lys	Val	Asn	Lys	Ala	Thr	1045	1050		1055
Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	Gln	Leu	Val	Tyr	Asp	Phe	1060	1065		1070
Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	Asp	Lys	Ile	Ala	Asp	Ile	1075	1080		1085
Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Gly	Asn	Met	1090	1095		1100
Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val	1105	1110		1115
Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr	1125	1130		1135
Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr	1140	1145		1150
Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr	1155	1160		1165
Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp	1170	1175		1180
Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala	1185	1190		1195
Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu	1205	1210		1215
Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp	Leu	Ser	Ser	Lys	Leu	Asn	1220	1225		1230
Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Lys	Phe	Leu	Asn	Gln	1235	1240		1245
Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	Ile	Pro	Tyr	Gly	Val	Lys	1250	1255		1260
Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	Asp	Ala	Leu	Leu	Lys	Tyr	1265	1270		1275
Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	Gln	Val	Asp	Arg	Leu	Lys	1285	1290		1295
Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	Ile	Pro	Phe	Gln	Leu	Ser	1300	1305		1310
Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser	Thr						1315	1320		

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<210> 107
 <211> 1329
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> PEPTIDE
 <222> (1)...(1329)
 <223> BoNT/A-BD-PAR4-Thrombin

<400> 107
 Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1 5 10 15
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
 20 25 30
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
 35 40 45
 Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
 50 55 60
 Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
 65 70 75 80
 Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
 85 90 95
 Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
 100 105 110
 Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
 115 120 125
 Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
 130 135 140
 Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
 145 150 155 160
 Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
 165 170 175
 Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
 180 185 190
 Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
 195 200 205
 Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
 210 215 220
 Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
 225 230 235 240
 Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
 245 250 255
 Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
 260 265 270
 Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
 275 280 285
 Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
 290 295 300
 Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
 305 310 315 320
 Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
 325 330 335
 Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
 340 345 350
 Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
 355 360 365
 Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr

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370	375	380
Thr Ile Tyr Asp Gly Phe Asn Leu Arg Asn Thr Asn Leu Ala Ala Asn		
385	390	395
Phe Asn Gly Gln Asn Thr Glu Ile Asn Asn Met Asn Phe Thr Lys Leu		400
	405	410
Lys Asn Phe Thr Gly Leu Phe Glu Phe Tyr Lys Leu Leu Cys Val Arg		415
	420	425
Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu Pro Arg Gly Tyr Pro Gly		430
	435	440
Gln Val Cys Ala Asn Asp Ser Asp Thr Leu Ala Leu Asn Asp Leu Phe		445
	450	455
Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn Leu Arg		460
465	470	475
Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser Lys Ile		480
	485	490
Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn Gln Ile		495
	500	505
Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu Lys Asn		510
	515	520
Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser Phe Trp		525
	530	535
Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn Glu Tyr		540
545	550	555
Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val Ser Leu		560
	565	570
Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu Ile Lys		575
	580	585
Gln Arg Val Val Phe Lys Tyr Ser Gln Met Ile Asn Ile Ser Asp Tyr		590
	595	600
Ile Asn Arg Trp Ile Phe Val Thr Ile Thr Asn Asn Arg Leu Asn Asn		605
	610	615
Ser Lys Ile Tyr Ile Asn Gly Arg Leu Ile Asp Gln Lys Pro Ile Ser		620
625	630	635
Asn Leu Gly Asn Ile His Ala Ser Asn Asn Ile Met Phe Lys Leu Asp		640
	645	650
Gly Cys Arg Asp Thr His Arg Tyr Ile Trp Ile Lys Tyr Phe Asn Leu		655
	660	665
Phe Asp Lys Glu Leu Asn Glu Lys Glu Ile Lys Asp Leu Tyr Asp Asn		670
	675	680
Gln Ser Asn Ser Gly Ile Leu Lys Asp Phe Trp Gly Asp Tyr Leu Gln		685
	690	695
Tyr Asp Lys Pro Tyr Tyr Met Leu Asn Leu Tyr Asp Pro Asn Lys Tyr		700
705	710	715
Val Asp Val Asn Asn Val Gly Ile Arg Gly Tyr Met Tyr Leu Lys Gly		720
	725	730
Pro Arg Gly Ser Val Met Thr Thr Asn Ile Tyr Leu Asn Ser Ser Leu		735
	740	745
Tyr Arg Gly Thr Lys Phe Ile Ile Lys Lys Tyr Ala Ser Gly Asn Lys		750
	755	760
Asp Asn Ile Val Arg Asn Asn Asp Arg Val Tyr Ile Asn Val Val Val		765
	770	775
Lys Asn Lys Glu Tyr Arg Leu Ala Thr Asn Ala Ser Gln Ala Gly Val		780
785	790	795
Glu Lys Ile Leu Ser Ala Leu Glu Ile Pro Asp Val Gly Asn Leu Ser		800
	805	810
Gln Val Val Val Met Lys Ser Lys Asn Asp Gln Gly Ile Thr Asn Lys		815
	820	825
Cys Lys Met Asn Leu Gln Asp Asn Asn Gly Asn Asp Ile Gly Phe Ile		830
	835	840
		845

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Gly	Phe	His	Gln	Phe	Asn	Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp	850	855	860
Tyr	Asn	Arg	Gln	Ile	Glu	Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp	865	870	875
Glu	Phe	Ile	Pro	Val	Asp	Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu	885	890	895
Ala	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	900	905	910
Ala	Leu	Val	Leu	Gln	Cys	Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	915	920	925
Ser	Pro	Ser	Glu	Asp	Asn	Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	930	935	940
Ile	Thr	Ser	Asp	Thr	Asn	Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	945	950	955
Asp	Leu	Ile	Gln	Gln	Tyr	Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	965	970	975
Glu	Asn	Ile	Ser	Ile	Glu	Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	980	985	990
Glu	Leu	Met	Pro	Asn	Ile	Glu	Arg	Phe	Pro	Asn	Gly	Lys	Lys	Tyr	Glu	995	1000	1005
Leu	Asp	Lys	Tyr	Thr	Met	Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	1010	1015	1020
His	Gly	Lys	Ser	Arg	Ile	Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	1025	1030	1035
Leu	Asn	Pro	Ser	Arg	Val	Tyr	Thr	Phe	Phe	Ser	Ser	Asp	Tyr	Val	Lys	1045	1050	1055
Lys	Val	Asn	Lys	Ala	Thr	Glu	Ala	Ala	Met	Phe	Leu	Gly	Trp	Val	Glu	1060	1065	1070
Gln	Leu	Val	Tyr	Asp	Phe	Thr	Asp	Glu	Thr	Ser	Glu	Val	Ser	Thr	Thr	1075	1080	1085
Asp	Lys	Ile	Ala	Asp	Ile	Thr	Ile	Ile	Ile	Pro	Tyr	Ile	Gly	Pro	Ala	1090	1095	1100
Leu	Asn	Ile	Gly	Asn	Met	Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	1105	1110	1115
Ile	Phe	Ser	Gly	Ala	Val	Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	1125	1130	1135
Ile	Pro	Val	Leu	Gly	Thr	Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	1140	1145	1150
Val	Leu	Thr	Val	Gln	Thr	Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	1155	1160	1165
Lys	Trp	Asp	Glu	Val	Tyr	Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	1170	1175	1180
Val	Asn	Thr	Gln	Ile	Asp	Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	1185	1190	1195
Glu	Asn	Gln	Ala	Glu	Ala	Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	1205	1210	1215
Gln	Tyr	Thr	Glu	Glu	Lys	Asn	Asn	Ile	Asn	Phe	Asn	Ile	Asp	Asp		1220	1225	1230
Leu	Ser	Ser	Lys	Leu	Asn	Glu	Ser	Ile	Asn	Lys	Ala	Met	Ile	Asn	Ile	1235	1240	1245
Asn	Lys	Phe	Leu	Asn	Gln	Cys	Ser	Val	Ser	Tyr	Leu	Met	Asn	Ser	Met	1250	1255	1260
Ile	Pro	Tyr	Gly	Val	Lys	Arg	Leu	Glu	Asp	Phe	Asp	Ala	Ser	Leu	Lys	1265	1270	1275
Asp	Ala	Leu	Leu	Lys	Tyr	Ile	Tyr	Asp	Asn	Arg	Gly	Thr	Leu	Ile	Gly	1285	1290	1295
Gln	Val	Asp	Arg	Leu	Lys	Asp	Lys	Val	Asn	Asn	Thr	Leu	Ser	Thr	Asp	1300	1305	1310
Ile	Pro	Phe	Gln	Leu	Ser	Lys	Tyr	Val	Asp	Asn	Gln	Arg	Leu	Leu	Ser			

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1315 1320 1325
 Thr

 <210> 108
 <211> 1323
 <212> PRT
 <213> Artificial Sequence

 <220>
 <221> PEPTIDE
 <222> (1)...(1323)
 <223> BoNT/A-BD-PAR4-Xa

 <400> 108
 Met Pro Phe Val Asn Lys Gln Phe Asn Tyr Lys Asp Pro Val Asn Gly
 1 5 10 15
 Val Asp Ile Ala Tyr Ile Lys Ile Pro Asn Ala Gly Gln Met Gln Pro
 20 25 30
 Val Lys Ala Phe Lys Ile His Asn Lys Ile Trp Val Ile Pro Glu Arg
 35 40 45
 Asp Thr Phe Thr Asn Pro Glu Glu Gly Asp Leu Asn Pro Pro Pro Glu
 50 55 60
 Ala Lys Gln Val Pro Val Ser Tyr Tyr Asp Ser Thr Tyr Leu Ser Thr
 65 70 75 80
 Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
 85 90 95
 Arg Ile Tyr Ser Thr Asp Leu Gly Arg Met Leu Leu Thr Ser Ile Val
 100 105 110
 Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
 115 120 125
 Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
 130 135 140
 Arg Ser Glu Glu Leu Asn Leu Val Ile Ile Gly Pro Ser Ala Asp Ile
 145 150 155 160
 Ile Gln Phe Glu Cys Lys Ser Phe Gly His Glu Val Leu Asn Leu Thr
 165 170 175
 Arg Asn Gly Tyr Gly Ser Thr Gln Tyr Ile Arg Phe Ser Pro Asp Phe
 180 185 190
 Thr Phe Gly Phe Glu Glu Ser Leu Glu Val Asp Thr Asn Pro Leu Leu
 195 200 205
 Gly Ala Gly Lys Phe Ala Thr Asp Pro Ala Val Thr Leu Ala His Glu
 210 215 220
 Leu Ile His Ala Gly His Arg Leu Tyr Gly Ile Ala Ile Asn Pro Asn
 225 230 235 240
 Arg Val Phe Lys Val Asn Thr Asn Ala Tyr Tyr Glu Met Ser Gly Leu
 245 250 255
 Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
 260 265 270
 Phe Ile Asp Ser Leu Gln Glu Asn Glu Phe Arg Leu Tyr Tyr Tyr Asn
 275 280 285
 Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
 290 295 300
 Gly Thr Thr Ala Ser Leu Gln Tyr Met Lys Asn Val Phe Lys Glu Lys
 305 310 315 320
 Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
 325 330 335
 Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp

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			340				345				350				
Asn	Phe	Val	Lys	Phe	Phe	Lys	Val	Leu	Asn	Arg	Lys	Thr	Tyr	Leu	Asn
			355				360				365				
Phe	Asp	Lys	Ala	Val	Phe	Lys	Ile	Asn	Ile	Val	Pro	Lys	Val	Asn	Tyr
			370				375				380				
Thr	Ile	Tyr	Asp	Gly	Phe	Asn	Leu	Arg	Asn	Thr	Asn	Leu	Ala	Ala	Asn
385				390				395				400			
Phe	Asn	Gly	Gln	Asn	Thr	Glu	Ile	Asn	Asn	Met	Asn	Phe	Thr	Lys	Leu
			405				410				415				
Lys	Asn	Phe	Thr	Gly	Leu	Phe	Glu	Phe	Tyr	Lys	Leu	Leu	Cys	Val	Arg
			420				425				430				
Gly	Ile	Ile	Thr	Ser	Lys	Thr	Lys	Ser	Leu	Ile	Glu	Gly	Arg	Gly	Tyr
			435				440				445				
Pro	Gly	Gln	Val	Ala	Leu	Asn	Asp	Leu	Phe	Thr	Glu	Tyr	Ile	Lys	Asn
			450				455				460				
Ile	Ile	Asn	Thr	Ser	Ile	Leu	Asn	Leu	Arg	Tyr	Glu	Ser	Asn	His	Leu
465				470				475				480			
Ile	Asp	Leu	Ser	Arg	Tyr	Ala	Ser	Lys	Ile	Asn	Ile	Gly	Ser	Lys	Val
			485				490				495				
Asn	Phe	Asp	Pro	Ile	Asp	Lys	Asn	Gln	Ile	Gln	Leu	Phe	Asn	Leu	Glu
			500				505				510				
Ser	Ser	Lys	Ile	Glu	Val	Ile	Leu	Lys	Asn	Ala	Ile	Val	Tyr	Asn	Ser
			515				520				525				
Met	Tyr	Glu	Asn	Phe	Ser	Thr	Ser	Phe	Trp	Ile	Arg	Ile	Pro	Lys	Tyr
			530				535				540				
Phe	Asn	Ser	Ile	Ser	Leu	Asn	Asn	Glu	Tyr	Thr	Ile	Ile	Asn	Cys	Met
545				550				555				560			
Glu	Asn	Asn	Ser	Gly	Trp	Lys	Val	Ser	Leu	Asn	Tyr	Gly	Glu	Ile	Ile
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Trp	Thr	Leu	Gln	Asp	Thr	Gln	Glu	Ile	Lys	Gln	Arg	Val	Val	Phe	Lys
			580				585				590				
Tyr	Ser	Gln	Met	Ile	Asn	Ile	Ser	Asp	Tyr	Ile	Asn	Arg	Trp	Ile	Phe
			595				600				605				
Val	Thr	Ile	Thr	Asn	Asn	Arg	Leu	Asn	Asn	Ser	Lys	Ile	Tyr	Ile	Asn
			610				615				620				
Gly	Arg	Leu	Ile	Asp	Gln	Lys	Pro	Ile	Ser	Asn	Leu	Gly	Asn	Ile	His
625				630				635				640			
Ala	Ser	Asn	Asn	Ile	Met	Phe	Lys	Leu	Asp	Gly	Cys	Arg	Asp	Thr	His
			645				650				655				
Arg	Tyr	Ile	Trp	Ile	Lys	Tyr	Phe	Asn	Leu	Phe	Asp	Lys	Glu	Leu	Asn
			660				665				670				
Glu	Lys	Glu	Ile	Lys	Asp	Leu	Tyr	Asp	Asn	Gln	Ser	Asn	Ser	Gly	Ile
			675				680				685				
Leu	Lys	Asp	Phe	Trp	Gly	Asp	Tyr	Leu	Gln	Tyr	Asp	Lys	Pro	Tyr	Tyr
			690				695				700				
Met	Leu	Asn	Leu	Tyr	Asp	Pro	Asn	Lys	Tyr	Val	Asp	Val	Asn	Asn	Val
705				710				715				720			
Gly	Ile	Arg	Gly	Tyr	Met	Tyr	Leu	Lys	Gly	Pro	Arg	Gly	Ser	Val	Met
			725				730				735				
Thr	Thr	Asn	Ile	Tyr	Leu	Asn	Ser	Ser	Leu	Tyr	Arg	Gly	Thr	Lys	Phe
			740				745				750				
Ile	Ile	Lys	Lys	Tyr	Ala	Ser	Gly	Asn	Lys	Asp	Asn	Ile	Val	Arg	Asn
			755				760				765				
Asn	Asp	Arg	Val	Tyr	Ile	Asn	Val	Val	Val	Lys	Asn	Lys	Glu	Tyr	Arg
			770												

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805																810				815			
Ser	Lys	Asn	Asp	Gln	Gly	Ile	Thr	Asn	Lys	Cys	Lys	Met	Asn	Leu	Gln								
			820				825						830										
Asp	Asn	Asn	Gly	Asn	Asp	Ile	Gly	Phe	Ile	Gly	Phe	His	Gln	Phe	Asn								
			835				840						845										
Asn	Ile	Ala	Lys	Leu	Val	Ala	Ser	Asn	Trp	Tyr	Asn	Arg	Gln	Ile	Glu								
			850				855						860										
Arg	Ser	Ser	Arg	Thr	Leu	Gly	Cys	Ser	Trp	Glu	Phe	Ile	Pro	Val	Asp								
865				870						875			880										
Asp	Gly	Trp	Gly	Glu	Arg	Pro	Leu	Ala	Leu	Ala	Gly	Gly	Gly	Gly	Ser								
			885						890			895											
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ala	Leu	Val	Leu	Gln	Cys								
			900						905			910											
Ile	Lys	Val	Asn	Asn	Trp	Asp	Leu	Phe	Phe	Ser	Pro	Ser	Glu	Asp	Asn								
			915									925											
Phe	Thr	Asn	Asp	Leu	Asn	Lys	Gly	Glu	Glu	Ile	Thr	Ser	Asp	Thr	Asn								
			930			935						940											
Ile	Glu	Ala	Ala	Glu	Glu	Asn	Ile	Ser	Leu	Asp	Leu	Ile	Gln	Gln	Tyr								
945				950						955			960										
Tyr	Leu	Thr	Phe	Asn	Phe	Asp	Asn	Glu	Pro	Glu	Asn	Ile	Ser	Ile	Glu								
			965						970			975											
Asn	Leu	Ser	Ser	Asp	Ile	Ile	Gly	Gln	Leu	Glu	Leu	Met	Pro	Asn	Ile								
			980						985			990											
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			995			1000						1005											
Phe	His	Tyr	Leu	Arg	Ala	Gln	Glu	Phe	Glu	His	Gly	Lys	Ser	Arg	Ile								
			1010			1015						1020											
Ala	Leu	Thr	Asn	Ser	Val	Asn	Glu	Ala	Leu	Leu	Asn	Pro	Ser	Arg	Val								
1025				1030						1035			1040										
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			1060						1065			1070											
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			1075			1080						1085											
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			1090			1095						1100											
Leu	Tyr	Lys	Asp	Asp	Phe	Val	Gly	Ala	Leu	Ile	Phe	Ser	Gly	Ala	Val								
1105				1110						1115			1120										
Ile	Leu	Leu	Glu	Phe	Ile	Pro	Glu	Ile	Ala	Ile	Pro	Val	Leu	Gly	Thr								
			1125						1130			1135											
Phe	Ala	Leu	Val	Ser	Tyr	Ile	Ala	Asn	Lys	Val	Leu	Thr	Val	Gln	Thr								
			1140						1145			1150											
Ile	Asp	Asn	Ala	Leu	Ser	Lys	Arg	Asn	Glu	Lys	Trp	Asp	Glu	Val	Tyr								
			1155			1160						1165											
Lys	Tyr	Ile	Val	Thr	Asn	Trp	Leu	Ala	Lys	Val	Asn	Thr	Gln	Ile	Asp								
			1170			1175						1180											
Leu	Ile	Arg	Lys	Lys	Met	Lys	Glu	Ala	Leu	Glu	Asn	Gln	Ala	Glu	Ala								
1185				1190						1195			1200										
Thr	Lys	Ala	Ile	Ile	Asn	Tyr	Gln	Tyr	Asn	Gln	Tyr	Thr	Glu	Glu	Glu								
			1205						1210			1215											
Lys	Asn	Asn	Ile	Asn	Phe	Asn	I																

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1265	1270	1275	1280
Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly Gln Val Asp Arg Leu Lys			
	1285	1290	1295
Asp Lys Val Asn Asn Thr Leu Ser Thr Asp Ile Pro Phe Gln Leu Ser			
	1300	1305	1310
Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser Thr			
	1315	1320	

<210> 109

<211> 4053

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(4050)

<223> Sequence encoding BoNT/A-ED-PAR1-Thrombin

<400> 109

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cggtcatttc ttctcaggaa cccaatgat aaatatgaac catttccatt tgtaataaaa 180
caatttaatt ataaagatcc tgtaaattgg gttgatattg cttatataaa aattccaaat 240
gcaggacaaa tgcaaccagt aaaagctttt aaaattcata ataaaatatg gggtattcca 300
gaaagagata catttacaaa tcctgaagaa ggagatttaa atccaccacc agaagcaaaa 360
caagttccag ttcatatta tgattcaaca tatttaagta cagataatga aaaagataat 420
tatttaaagg gagttacaaa attatttgag agaatttatt caactgatct tggaagaatg 480
ttgttaacat caatagtaag gggaatacca ttttggggtg gaagtacaat agatacagaa 540
ttaaaagtta ttgatactaa ttgtattaat gtgatacaac cagatggtag ttatagatca 600
gaagaactta atctagtaat aataggaccc tcagctgata ttatacagtt tgaatgtaaa 660
agctttggac atgaagtttt gaatcttac cgaaatggtt atggctctac tcaatacatt 720
agatttagcc cagattttac atttggtttt gaggagtcac ttgaagttga tacaatcct 780
cttttaggtg caggcaaatt tgctacagat ccagcagtaa cattagcaca tgaacttata 840
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gaaattaata atatgaattt tactaaacta aaaaatttta ctggattgtt tgaattttat 1440
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gatccaatag ataaaaatca aattcaatta ttttaatttag aaagtagtaa aattgaggta 2940
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<210> 110

<211> 4029

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(4026)

<223> Sequence encoding BoNT/A-ED-PAR1-Xa

<400> 110

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cggtcatttc ttctcaggaa cccattttgt aataaacaat ttaattataa agatcctgta 180
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acagatccag cagtaacatt agcacatgaa cttatacatg ctggacatag atttatatgga 840
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<210> 111

<211> 4038

<212> DNA

<213> Artificial Sequence

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<221> mat_peptide

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<222> (1)...(4035)

<223> Sequence encoding BoNT/A-ED-PAR2-Trypsin

<400> 111

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Li *et al.*, Degradable Clostridial Toxins

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<210> 112

<211> 4014

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(4011)

<223> Sequence encoding BoNT/A-ED-PAR2-Xa

<400> 112

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ccagatggta gttatagatc agaagaactt aatctagtaa taataggacc ctcagctgat 600
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ggaaaaaagt atgagttaga taaatatact atgttccatt atcttcgtgc tcaagaattt 1800
gaacatggta aatctaggat tgctttaaca aattctgtta acgaagcatt attaaatcct 1860

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Li et al., Degradable Clostridial Toxins

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gaagtaagta  ctacggataa  aattgcggat  ataactataa  ttattccata  tataggacct  2040
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<210> 113

<211> 4044

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(4041)

<223> Sequence encoding BoNT/A-ED-PAR3-Thrombin

<400> 113

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Li *et al.*, Degradable Clostridial Toxins

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Li *et al.*, Degradable Clostridial Toxins

<211> 4020

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(4017)

<223> Sequence encoding BoNT/A-ED-PAR3-Xa

<400> 114

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gatttaaatc caccaccaga agcaaaacia gttccagttt catattatga ttcaacatat 360
ttaagtacag ataataaaaa agataattat ttaaggaggag ttacaaaatt atttgagaga 420
atattattcaa ctgatcttgg aagaatgttg ttaacatcaa tagtaagggg aataccattt 480
tgggggtggaa gtacaataga tacagaatta aaagttattg atactaattg tattaatgtg 540
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<210> 115

<211> 4071

<212> DNA

<213> Artificial Sequence

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<221> mat_peptide

<222> (1)...(4068)

<223> Sequence encoding BoNT/A-ED-PAR4-Thrombin

<400> 115

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<213> Artificial Sequence

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<223> Sequence encoding BoNT/A-ED-PAR4-Xa

<400> 116

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ccagtttcat attatgattc aacatattta agtacagata atgaaaaaga taattattta 420

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<213> Artificial Sequence

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```

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Li *et al.*, Degradable Clostridial Toxins

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taa 3903

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<211> 3921

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(3918)

<223> Sequence encoding BoNT/A-TD-PAR3-Thrombin

<400> 121

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ccaccaccag aagcaaaaca agttccagtt tcatattatg attcaacata tttaaagtaca 240
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<211> 3903

<212> DNA

<213> Artificial Sequence

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<221> mat_peptide

<222> (1)...(3900)

<223> Sequence encoding BoNT/A-TD-PAR3-Xa

<400> 122

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<210> 123
 <211> 3921
 <212> DNA
 <213> Artificial Sequence

<220>
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 <222> (1)...(3918)
 <223> Sequence encoding BoNT/A-TD-PAR4-Thrombin

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<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(3900)

<223> Sequence encoding BoNT/A-TD-PAR4-Xa

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<220>

<221> mat_peptide

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<223> Sequence encoding BoNT/A-BD-PAR1-Thrombin

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 <213> Artificial Sequence

<220>
 <221> mat_peptide
 <222> (1)...(3966)
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<213> Artificial Sequence

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<221> mat_peptide

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<223> Sequence encoding BoNT/A-BD-PAR2-Trypsin

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<213> Artificial Sequence

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<223> Sequence encoding BoNT/A-BD-PAR2-Xa

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<223> Sequence encoding BoNT/A-BD-PAR3-Xa

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<212> DNA

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<220>

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<222> (1)...(3984)

<223> Sequence encoding BoNT/A-BD-PAR4-Thrombin

<400> 131

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<211> 3969

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)... (3966)

<223> Sequence encoding BoNT/A-BD-PAR4-Xa

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<210> 135

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<211> 6
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<210> 137

<211> 4029

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(4026)

<223> Expression optimized sequence encoding
BoNTA-ED-PAR1FactorXa

<400> 137

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tccacctacc tgteccaccg caacgagaag gacaactacc tgaaaggcgt taccaaactg 420
ttcgaacgta tctactccac cgacctgggc cgtatgctgc tgactagcat cgttcgtggc 480
atcccggttc gggggcggtc caccatcgac accgaactga aagttatcga caccaactgc 540
atcaacgta tccagccgga cggctcctac cgttcggaag aactgaacct gggttatcat 600
ggcccgctcg ctgacatcat ccagttcgaa tgcaaatcct tcggccacga agttctgaac 660

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Li *et al.*, Degradable Clostridial Toxins

```

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Li *et al.*; Degradable Clostridial Toxins

<211> 4038

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(4035)

<223> Expression optimized sequence encoding

BoNTA-ED-PAR2trypsin

<400> 138

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Li *et al.*, Degradable Clostridial Toxins

```

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<210> 139

<211> 4014

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(4011)

<223> Expression optimized sequence encoding

BoNTA-ED-PAR2FactorXa

<400> 139

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Li *et al.*, Degradable Clostridial Toxins

```

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<211> 4044

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(4041)

<223> Expression optimized sequence encoding
BoNTA-ED-PAR3thrombin

<400> 140

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Li et al., Degradable Clostridial Toxins

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```

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<210> 141

<211> 4020

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(4017)

<223> Expression optimized sequence encoding
BoNTA-ED-PAR3FactorXa

<400> 141

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Li *et al.*, Degradable Clostridial Toxins

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<210> 142

<211> 4071

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(4068)

<223> Expression optimized sequence encoding
BoNTA-ED-PAR4thrombin

<400> 142

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- Li et al., Degradable Clostridial Toxins

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<210> 143

<211> 4047

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(4044)

<223> Expression optimized sequence encoding
BoNTA-ED-PAR4FactorXa

Li *et al.*, Degradable Clostridial Toxins

<400> 143

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Li et al., Degradable Clostridial Toxins

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<211> 3921

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(3918)

<223> Expression optimized sequence encoding
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<400> 144

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Li et al., Degradable Clostridial Toxins

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<210> 145

<211> 3903

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(3900)

<223> Expression optimized sequence encoding

BoNTA-TD-PAR1FactorXa

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Li *et al.*, Degradable Clostridial Toxins

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<210> 146

<211> 3921

<212> DNA

<213> Artificial Sequence

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<221> mat_peptide

Li et al., Degradable Clostridial Toxins

<222> (1)...(3918)

<223> Expression optimized sequence encoding
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<400> 146

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```


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```

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<210> 147

<211> 3903

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(3900)

<223> Expression optimized sequence encoding
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<400> 147

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```

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<210> 148

<211> 3921

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(3918)

<223> Expression optimized sequence encoding
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<400> 148

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<210> 149

<211> 3903

<212> DNA

<213> Artificial Sequence

Li *et al.*, Degradable Clostridial Toxins

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<221> mat_peptide

<222> (1)...(3900)

<223> Expression optimized sequence encoding
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Li *et al.*, Degradable Clostridial Toxins

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taa 3903

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<210> 150

<211> 3921

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(3918)

<223> Expression optimized sequence encoding

BoNTA-TD-PAR4thrombin

<400> 150

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gtttacgact tcaccgacga aacctccgaa gtttccacca ccgacaaaat cgctgacatc 1920

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Li *et al.*, Degradable Clostridial Toxins

```

accatcatta tcccgtacat cggcccggct ctgaacatcg gcaacatgct gtacaaagac 1980
gacttcggtg gcgctctgat cttctccggc gctgttatcc tgctggaatt catcccggaa 2040
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<210> 151

<211> 3903

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(3900)

<223> Expression optimized sequence encoding
BoNTA-TD-PAR4FactorXa

<400> 151

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gacaacgaga aggacaacta cctgaaaggc gttaccaaac tgttcgaacg tatctactcc 300
accgacctgg gccgtatgct gctgacctcc atcgttcgtg gcatcccgtt ctggggcggc 360
tccaccatcg acaccgaact gaaagtattc gacaccaact gcatcaacgt tatccagccg 420
gacggctcct accgttccga agaactgaac ctggttatca tcggcccgtc cgctgacatc 480
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Li et al., Degradable Clostridial Toxins

ctggctcagc	aactgatcca	cgctggccac	cgtctgtacg	gcatcgctat	caacccgaac	720
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taa						3903

<210> 152
 <211> 3990
 <212> DNA

Li *et al.*, Degradable Clostridial Toxins

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(3987)

<223> Expression optimized sequence encoding

BoNTA-BD-PAR1Thrombin

<400> 152

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Li et al., Degradable Clostridial Toxins

```

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<211> 3972

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(3969)

<223> Expression optimized sequence encoding
BoNTA-BD-PAR1FactorXa

<400> 153

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<210> 154

<211> 3990

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(3987)

<223> Expression optimized sequence encoding

BoNTA-BD-PAR2trypsin

<400> 154

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3990

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<211> 3972

<212> DNA

<213> Artificial Sequence

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<221> mat_peptide

<222> (1)...(3969)

<223> Expression optimized sequence encoding
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<400> 155

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<211> 3990

<212> DNA

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<221> mat_peptide

<222> (1)...(3987)

<223> Expression optimized sequence encoding
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Li *et al.*, Degradable Clostridial Toxins

```

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<210> 157

<211> 3972

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(3969)

<223> Expression optimized sequence encoding

BoNTA-BD-PAR3FactorXa

<400> 157

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<210> 158

<211> 3990

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(3987)

<223> Expression optimized sequence encoding
BoNTA-BD-PAR4thrombin

<400> 158

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Li *et al.*, Degradable Clostridial Toxins

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<210> 159

<211> 3972

<212> DNA

<213> Artificial Sequence

<220>

<221> mat_peptide

<222> (1)...(3969)

<223> Expression optimized sequence encoding
BoNTA-BD-PAR4FactorXa

<400> 159

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Li *et al.*, Degradable Clostridial Toxins

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1

5

A. CLASSIFICATION OF SUBJECT MATTER
C07K14/33 C07K14/705

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	US 2002/137886 A1 (LIN WEI-JEN ET AL) 26 September 2002 (2002-09-26) cited in the application page 2, column 1, paragraph 15 - paragraph 17	1-61
A	----- US 6 168 932 B1 (UCKUN FATIH M ET AL) 2 January 2001 (2001-01-02) column 1, line 23 - line 50	1-61
A	----- US 2003/124147 A1 (VALLERA DANIEL A ET AL) 3 July 2003 (2003-07-03) page 1, paragraph 5 - paragraph 6 ----- -/--	1-61

☒ Further documents are listed in the continuation of box C

☒ Patent family members are listed in annex.

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- *A* document defining the general state of the art which is not considered to be of particular relevance
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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search

12 January 2006

Date of mailing of the international search report

24/01/2006

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Authorized officer

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	<p>PASTAN I ET AL: "PSEUDOMONAS EXOTOXIN: CHIMERIC TOXINS" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOCHEMICAL BIOLOGISTS, BIRMINGHAM,, US, vol. 264, no. 26, 15 September 1989 (1989-09-15), pages 15157-15160, XP000048580 ISSN: 0021-9258 page 15159, column 1, line 24 - page 15160, column 1, line 42</p>	1-61
A	<p>TSUI J K C: "BOTULINUM TOXIN AS A THERAPEUTIC AGENT" PHARMACOLOGY AND THERAPEUTICS, ELSEVIER, GB, vol. 72, no. 1, 1996, pages 13-24, XP001179766 ISSN: 0163-7258 page 20, column 2, line 4 - page 21, column 1, line 18</p>	1-61
A	<p>COUGHLIN S R: "Thrombin signalling and protease-activated receptors" NATURE, NATURE PUBLISHING GROUP, LONDON, GB, vol. 407, no. 6801, 14 September 2000 (2000-09-14), pages 258-264, XP002981969 ISSN: 0028-0836 the whole document</p>	1-61
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			EP 1381677 A2	21-01-2004
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US 6168932	B1	02-01-2001	NONE	
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